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A CONTRIBUTION TO THE EMBRYOLOGY OF THE GENUS *PORTULACA*

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Introduction

Most embryological investigations on the flowering plants during the past have been concerned with the development of the gametophytes, particularly the embryo-sac. The study of embryonal development has received comparatively little attention. Thus although the development of the embryo-sac and pollen is known in several genera of the Portulacaceæ,—*Calandrinia, Claytonia, Portulaca, Talimum, Montia* and *Anacampseros* (Schnarf, 1931)—, the details of embryo development till recently had not been worked out even in one species. Only when the present investigation had been nearly completed, there has appeared a paper by Souèges (1938) describing the embryo development in *Portulaca oleracea.*

During the course of the investigation the writer has studied the development of endosperm and embryo in *Portulaca grandiflora* Hook., *P. oleracea* Linn. and *P. quadrifida* Linn. In addition to this the development of pollen and embryo-sac in *Portulaca quadrifida*, a species which has hitherto received no attention, is also described.

The material of all the species was collected from plants growing in the Botanical Garden of the Benares Hindu University. *Portulaca grandiflora* is a common garden plant, while *P. oleracea* and *P. quadrifida* are common weeds throughout the country. Fixationsr were made in the morning between 7 to 10 A.M. during the winter season. This time is quite suitable for getting the mitotic divisions in the proembryo. Three different fixatives were used, namely, Nawaschin's fluid, Formalin-acetic-alcohol and Carnoy's fluid. The first two fixatives proved quite satisfactory. Depending upon the stage of development, sections were cut at a thickness ranging from 8 to 14μ For the study of embryo development ovules were dissected out of

the gynœcium and sectioned individually. Delafield's Hæmatoxylin, Heidenhain's Iron-alum Hæmatoxylin, Ehrlich's Hæmatoxylin and a combination of Safranin and Gentian violet were used as stains. The last combination is more satisfactory than others for the study of seed-coat development, particularly during the early stages, the grains deposited in the layers of the testa looking bright violet. As recommended by Cooper (1935), Ehrlich's Hæmatoxylin was used for the study of pollen grains, which are rich in starch grains. But it was not found so satisfactory. In order to remove the starch grains, slides were treated with aqueous solution of Taka Diastase and then stained. This slightly improved the staining. The external characters of the pollen grains were studied in Methylgreen glycerine-jelly mounts.

MICROSPOROGENESIS

The development of the anther and pollen in *Portulaca grandiflora* has been worked out by Dahlgren (1916), Rócen (1927) and Tjebbes (1928). Rócen (1927) has also investigated *Portulaca oleracea*. He found that it does not differ much from *Portulaca grandiflora* in the development of floral structures. The development of the male gametophyte in the former species has been further described by Cooper (1935). This aspect of the life-history, therefore, has been studied by the present author only in *Portulaca quadrifida*.

The anther is four-lobed. The primary archesporium in each lobe is confined to a single hypodermal row of four to six cells (Fig. 1). Each archesporial cell cuts off a parietal cell towards the outside and the primary sporogenous cell towards the inside (Fig. 1). The primary sporogenous cells directly function as the pollen-mother cells, as described by Cooper (1935) in *Portulaca oleracea* (Figs. 2 and 3). From the observations of Rócen (1927) on other genera it appears that this feature is characteristic of the Portulacaceæ. The pollen-mother cells even from the beginning are much bigger than the surrounding cells (Fig. 1). An unusual feature is the frequent occurrence of vacuoles in the protoplasm of the pollen-mother cells (Figs. 2 and 3). The cells of the primary parietal layer divide anticlinally and periclinally to form two layers (Fig. 2). One adjacent to the spore-mother cells divides once more periclinally (Fig. 3). In this way the primary parietal layer forms three layers. Out of these three layers, the middle one is crushed early during further development. The layer in the hypodermal position develops into the fibrous endothecium. The innermost parietal layer forms the tapetum. During the reduction division of the pollen-mother cells the single nucleus of the tapetal cells divides once mitotically and the cells thus become two-nucleate. In the material examined, they were neither observed to become multinucleate, nor their nuclei were seen to divide amitotically, as has been reported by Rócen (1927) in *Portulaca grandiflora*. The tapetal cells during their entire life were never seen to leave their place to form any periplasmodium. They thus appear to be purely secretory in function. Granular cutinisation of the inner surface of the

tapetal and the fibrous endothecial cells has been observed as in several other Centrospermales (Kajale, 1940 *b*).

During the first meiotic division the spindle fibres in the pollen-mother cells are surrounded by a dense zone of cytoplasm as reported by Cooper (1935) in *Portulaca oleracea* (Fig. 4). At the same time each pollen-mother cell is surrounded by a distinct sheath of mucilage, which appears between the protoplasm and its original wall (Fig. 4). It persists till the pollen-mother cell divides to form the tetrad. After the disintegration of this sheath and the original cell wall the four pollen grains separate off from one another. A large percentage of them are arranged in a tetrahedral manner, while intermixed with the latter a few are seen to form isobilateral tetrads.

The young pollen grain is rich in protoplasm. Very soon it develops the exine and intine, and at the same time it begins to increase in size. As a consequence of this enlargement it becomes vacuolate (Fig. 5). The nucleus along with some cytoplasm is pushed to the periphery. Here it divides mitotically into two nuclei, which are separated from each other by a curved wall. Thus the generative cell is organised (Fig. 6). It is interesting to note that in a few instances small vacuoles were observed in the tube nucleus (Fig. 6). The behaviour of the generative cell in *Portulaca quadrifida* is seen to differ from the angiosperms in general. In the flowering plants generally this cell is ephemeral, the new wall disappearing almost as soon as it is formed. It is, therefore, not possible for the cell to grow in size or change its shape. In the present material, however, the generative cell increases in size along with the increase in the dimensions of the pollen grain (compare Figs. 6 to 8). Its form varies with the plane in which it is cut (Figs. 7 and 8). It also stains slightly deeper than the other parts. The generative cell, however, does not persist till the shedding time of the pollen grain. Before this stage is reached, the cell wall of the generative cell disappears. The nucleus divides. This is followed by the division of its cytoplasm and two somewhat elongated male cells with a distinct sheath of cytoplasm surrounding the nucleus are formed. Male cells have also been reported in *Portulaca oleracea* by Cooper (1935) and have been seen by the writer in *Portulaca grandiflora*.

Each pollen grain as usual has intine and exine, the former lining the latter all round from inside. The thickness of the intine differs in the three species. It is thicker in *Portulaca oleracea* than in the other two species and is slightly thinner than the exine.

The exine is perforated generally by 30 furrows so arranged as to form twelve exactly similar pentagons (Fig. 9). Fischer (1890) has described a similar figure of the pollen grains of *Portulaca oleracea* and *P. grandiflora*. Franz (1908) has also pointed out that the dodecahedron is the usual configuration for the microspores in a large number of plants of this family. The length of the germinal furrows varies in the different species. They are comparatively narrower and longer in *P. quadrifida* than in the other two species.

1*a*
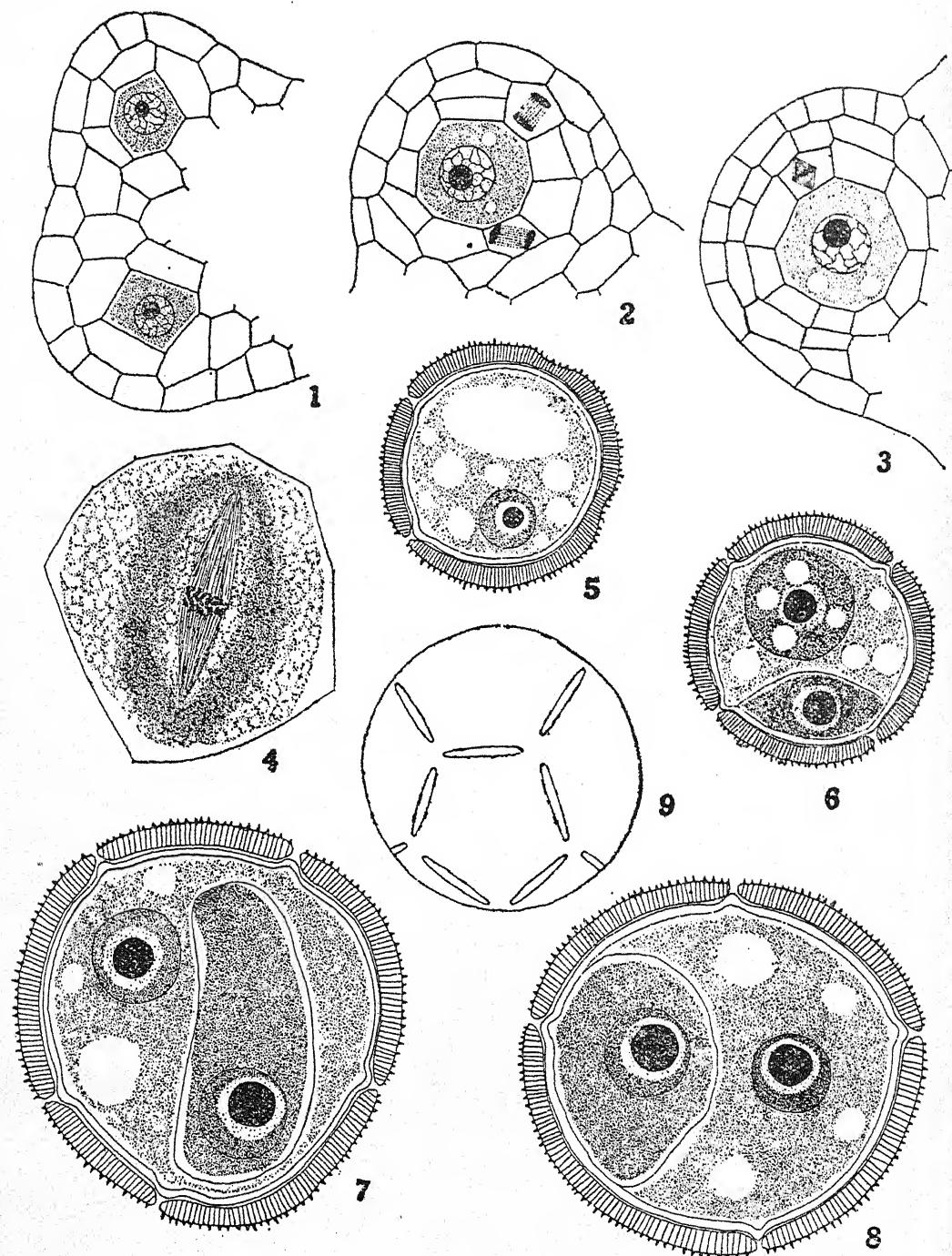
Figs. 1–9. *Portulaca quadrifida.* Figs. 1–3. Transverse sections of anther-lobes at various stages of development, In Figs. 2 and 3 vacuoles

In *P. grandiflora* they are quite broad and the length is only about 2–3 times the maximum breadth. They are thus roughly ellipsoidal in outline. The germinal furrows do not possess any germ pore and themselves function as the passage for the outgoing pollen tube.

The structure of the exine is just the same as described by the author for members of the Amarantaceæ (Kajale, 1940 *b*). As seen in section, the exine is found to be composed of alternating light and dark staining portions, the latter appearing rod-like (Figs. 5 to 8). During the later development of the pollen grain the exine gets thinner than before. The cause of this change is discussed by the author elsewhere (Kajale, 1940 *b*).

The structure of exine is further interesting in that it bears numerous spines on its outside. The spines in different species differ in their length. In general they are bigger in *P. grandiflora* than in the other species. The smallest spines are observed in *P. quadrifida*, while *P. oleracea* is intermediate between the two species so far as this character is concerned. In *P. quadrifida* the spines are so small that they may be easily overlooked.

The pollen grains are shed at the three-nucleate stage as in the Centrospermales in general. Their diameter is 75 to 80 μ in *Portulaca grandiflora*, 73 to 77 μ in *P. oleracea* and 66 to 70 μ in *P. quadrifida*.

STRUCTURE OF THE OVARY AND OVULE

The ovary is semi-inferior in the genus. It is generally tri-locular (Figs. 10 and 12), but sometimes more loculi may be seen. It develops from the tip of the floral axis after the other parts have been differentiated. Along with the development of the ovary wall septa equal to the number of the loculi begin to develop from the wall. The ovules develop from the septa as lateral outgrowths (Fig. 10). The septa meet in the centre of the gynœcium resulting in the axile placentation (Fig. 10). The fusion of the septa takes place throughout the length of the gynœcium, unlike in *Sesuvium Portulacastrum* (Kajale, 1940 *a*), where the septa do not meet in the upper part. The entire central axis, however, does not bear ovules. The latter are confined mostly to lower half of the placentas, while the upper half is barren (Fig. 11). Further the upper part does not persist during the development of the fruit (Figs. 11 and 13). Consequently the axile placentation becomes almost free central during the later stages (Fig. 11).

The development of the ovule has been studied in *Portulaca quadrifida*. The first indication of the ovule is seen in the form of

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are seen in the cytoplasm of the pollen-mother cells. Fig. 4. A pollen-mother cell in the metaphase of the I meiotic division; a perinuclear zone is seen round the spindle. Fig. 5. 1-nucleate pollen grain. Figs. 6–8. 2-nucleate pollen grains. Generative cell increases in size and presents different shape when cut in different planes. Fig. 9. A pollen grain in surface view showing the arrangement of the furrows. (Figs. 1–3 and 5–8 × 450; Fig. 4 × 800; Fig. 9 × 250.)
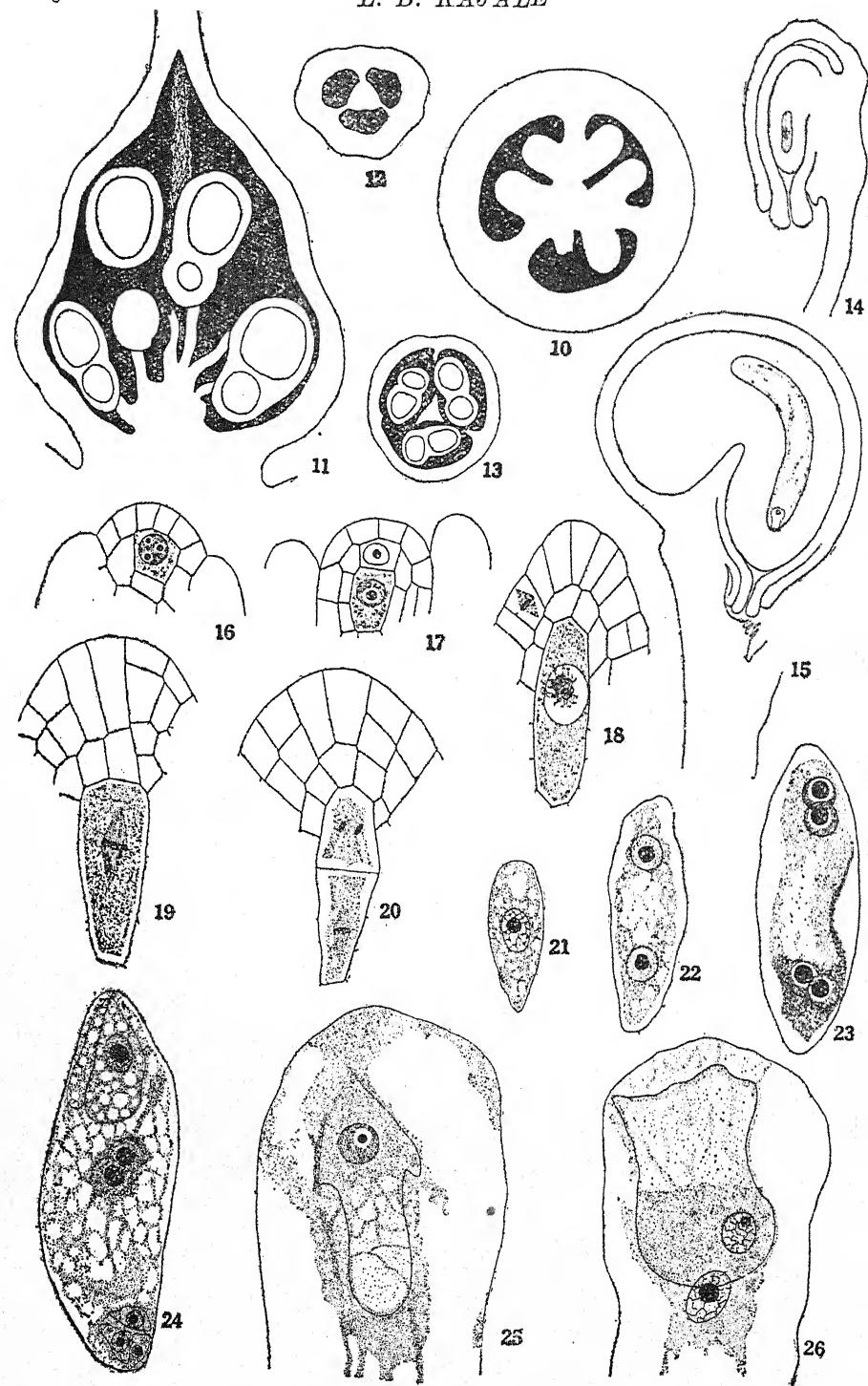
Figs. 10–26. *Portulaca quadrifida*. Fig. 10. Transverse section of a ovary showing the developing ovules and their arrangement. Fig. 11. Longitudinal section of the ovary with ovules at the mature embryo-sac

a small protuberance on the margins of the ingrowing septa (Fig. 10). It soon becomes bent in the upper part due to pronounced unilateral growth. The archesporium appears about this stage and the two parts, namely, the comparatively narrow funicle and the ovule proper become distinct. The funicle in the mature ovule is fairly long and erect (Fig. 15). Each ovule possesses two integuments which appear as annular outgrowths from the base of the nucellus. The differentiation of the integuments in the ovule is almost synchronous with the development of the archesporium (Fig. 16). By the time the megaspore-mother cell is well differentiated the ovule has developed to a sufficient extent to assume nearly an anatropous form (Fig. 14). The integuments by this time have covered almost the entire nucellus. In between them at the chalazal end a prominent air-space is observed (Fig. 14), a feature which appears to be characteristic of the whole order Centrospermales. Both the integuments do not take part in the formation of the micropyle. As in the Centrospermales in general, the inner integument grows ahead of the outer and alone forms the micropyle (Fig. 15). The integuments are mostly two cells thick, except at the micropylar extremity, where they are thicker.

The nucellus about the megaspore-mother cell stage is two layers thick at the apex, three to five layers thick at the sides, and nine to ten layers thick below the megaspore-mother cell. Along with the development of the female gametophyte, the nucellus also develops further, particularly in the chalazal region. The epidermal cap over the nucellus is formed in a characteristic manner. Those cells situated just below the micropyle merely stretch out radially, while the surrounding cells divide by periclinal walls (Figs. 18 to 20). This corresponds to what has been observed in several genera of the Ficoideæ, namely, *Mesembrianthemum* (Schmid, 1925), *Trianthema* (Bhargava, 1935) and *Sesuvium* (Kajale, 1940 a).

MEGASPOROGENESIS

Simultaneously with the development of the integumental initials one cell of the nucellus just below the epidermis begins to take a deeper stain than the rest, increases in size and differentiates

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stage. Dotted part represents the part of the central axis that disappears during further development. Figs. 12–13. Transverse sections of the ovary at the apex and base respectively about the time of fertilisation. Figs. 14–15. Ovules at 1-nucleate and mature embryo-sac stages. Fig. 16. A young nucellus showing a single archesporial cell. Fig. 17. Another nucellus showing a megaspore-mother cell. Fig. 18. A megaspore-mother cell during the prophase of the I meiotic division. Fig. 19. A megaspore-mother cell in the I metaphase. Excluding the central cells other cells of the nucellar epidermis have divided periclinally to form an epidermal cap. This is also shown in next figure. Fig. 20. Dyad dividing. Figs. 21–23. Various stages in the development of the embryo-sac. Fig. 24. 7-Nucleate embryo-sac showing two synergids, secondary nucleus and three antipodals. Fig. 25. Micropylar part of a mature embryo-sac showing one of the synergids. Fig. 26. The same showing the egg and one polar nucleus. (Fig. 10 × 100; Figs. 11–13 × 25; Fig. 14 × 100; Fig. 15 × 75; Figs. 16–26 × 450.)

as the primary archesporial cell (Fig. 16). It cuts off a parietal cell (Fig. 17). The resulting megaspore-mother cell increases in volume until it has reached the specific maximum size (Fig. 18). Now its nucleus prepares for the meiotic divisions. These are quite normal. After the first division a cell wall is formed and two dyad cells are organized (Figs. 19 to 20). The first meiotic spindle is present in the upper part of the megaspore-mother cell (Fig. 19). Hence the micropylar dyad cell is generally smaller than the chalazal one. Both of them undergo the II meiotic division almost simultaneously and a linear row of four megaspores is formed. At times the spindle in the micropylar dyad is arranged at right angles to that of the lower dyad (Fig. 20). Such an arrangement of the spindles leads to the formation of a T-shaped tetrad. Of the four megaspores only the chalazal one develops into the embryo-sac. The other three towards the micropyle degenerate very rapidly.

The uni-nucleate embryo-sac may be very much vacuolate (Fig. 21), or may possess a few vacuoles on either side of the nucleus. Its nucleus divides mitotically into two. The daughter nuclei travel towards the two poles of the embryo-sac and on reaching there divide once again to form a four-nucleate embryo-sac (Figs. 22 and 23). These four nuclei divide once again mitotically and a typical eight-nucleate embryo-sac is formed.

The egg-apparatus is organized in the normal manner. The egg is a broad flask-shaped structure. Its micropylar end is vacuolate. The nucleus with cytoplasm is present in the basal region (Fig. 26). Some difference in the size of the egg in different plants has been frequently observed (Figs. 26 and 28). The synergids are two in number. The nucleus is present in the upper part from the very beginning (Fig. 24). They have prominent hooks and the nucleus may be situated near the hooks or slightly above them (Figs. 25 and 27). The young synergids are prominently vacuolate, the vacuoles being present all over the synergids (Fig. 24). It appears that as the synergids become mature these vacuoles fuse to form a large basal vacuole (Fig. 25). As in the case of eggs a difference in the size of the synergids in different plants is also observed (compare Figs. 25 and 27). The antipodals are three small cells occupying the chalazal end of the embryo-sac (Fig. 24). Sooner or later they degenerate and no trace of them is seen during the embryo development. Out of the two polar nuclei the lower one moves towards the micropylar end of the embryo-sac, so that both the polar nuclei come to lie closely pressed against each other somewhere in the vicinity of the egg (Figs. 26 and 27). During this time they also show an increase in size and are bigger than any other nuclei in the embryo-sac. Their further fate is described below.

FERTILISATION

The details of fertilisation have been studied in *Portulaca quadrifida* only. The pollen grains reach the stigma in the three-nucleate condition. There they begin to germinate rather quickly. The ends of pollen tubes can be clearly seen inside the embryo-sac

of flowers fixed within an hour or two from the time of their opening. Artificial culture of the pollen grains also shows the same behaviour. It has been found that the pollen grains can germinate very easily even in tap water. When they are placed in a drop of water, they burst almost immediately and the pollen tubes come out in no time.

A large number of pollen grains germinate on the stigma and hence many pollen tubes can be observed on their way to the ovules inside the style. Further down they make their way through one of the placentas until they reach an ovule. Now they pass through the micropyle into the nucellus and penetrating it they break through the apex of the embryo-sac.

The end of the pollen tube on entering the embryo-sac swells up enormously, so much so that it fills the greater part of the micro-
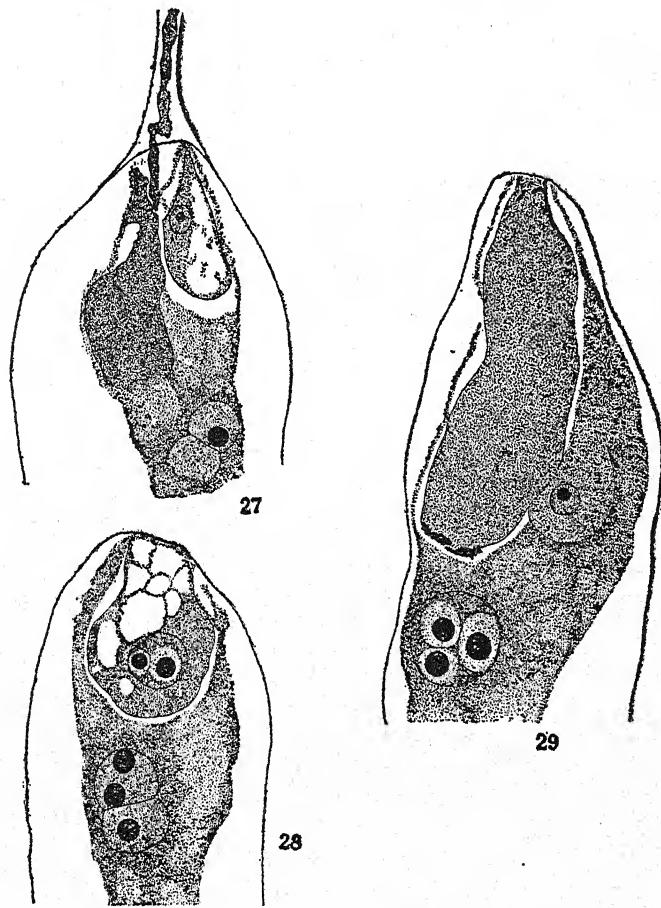
Figs. 27–29. *Portulaca quadrifida.* Fig. 27. Micropylar part of an embryo-sac showing one synergid, remains of the pollen tube and two polar nuclei. Fig. 28. The same showing the fusion of the male gametes with the egg nucleus and the polar nuclei. Fig. 29. The same showing the remains of the pollen tube, egg and triple fusion (× 450).

pylar end of the embryo-sac (Figs. 27 and 29). On its way through the embryo-sac the pollen tube passes along one of the synergids, which as a result is destroyed completely, while the other synergid, as in *Salix* (Chamberlain, 1897), *Silphium* (Merrel, 1900), etc., is left intact (Fig. 27). Some times, however, both the synergids were seen to be destroyed by the entrance of the pollen tube. The extreme tip of the pollen tube extends beyond the lower end of the egg and there its contents are discharged (Figs. 27 and 29).

Out of the two gametes brought by the pollen tube one fuses with the egg. It rounds up and passing inside the egg unites with its nucleus. Just before the fusion is complete a round deeply staining body is seen inside the nucleus of the egg (Fig. 28). Ultimately it fuses with the nucleolus of the egg. Unlike in the large majority of angiosperms, the polar nuclei do not fuse to form a secondary nucleus before fusing with the male gamete. They remain separate till the male gamete reaches them (Fig. 28). The triple fusion then takes place almost simultaneously between the two polar nuclei and the second male gamete (Figs. 28 and 29). The individual boundaries of the three nuclei disappear and three nucleoli are seen enclosed within a common nuclear membrane (Fig. 29). They later fuse to form a big triploid nucleus. The second male gamete also undergoes change in shape like the one fusing with the egg.

THE EMBRYO

This phase of life-history has been studied in all the three species, but *Portulaca quadrifida* has been studied more critically.

The fertilised egg first undergoes a transverse division (Figs. 30, 31 and 61). The two cells formed as a result of this division behave differently during further development. The micropylar cell generally divides before the other (Figs. 32 and 62). It may divide once or twice to form up to three cells before any transverse division takes place in the apical cell (Figs. 32, 63 and 64). At times it may not divide also (Fig. 38). This cell, therefore, varies a good deal in its behaviour. The transverse divisions in the apical cell follow a certain definite sequence. First it divides transversely (Figs. 33 and 64). The resulting apical cell does not divide any more in a transverse plane for some time, while the penultimate cell divides transversely to form two cells as shown in Fig. 34. The one adjacent to the apical cell does not divide again transversely, while the other divides into two cells (Figs. 36, 39 and 65). Next transverse division takes place in the fourth cell from the apex (Fig. 54). By now four cells result from the apical cell of the two-celled proembryo. In this way depending upon the behaviour of the micropylar cell a proembryo of five to six or sometimes seven cells is organized in *P. quadrifida* (Figs. 35 and 36). A similar organization of the proembryo is observed in *P. grandiflora*. This will be clear from Figs. 61 to 65. Some stages were observed in *P. oleracea* also and they leave no doubt that the proembryo in that species also is organized on the same lines. The length of the proembryo in the

latter two species is also five to six cells as in *P. quadrifida* (Figs. 52 and 66).

Out of five to seven cells of the proembryo the embryo proper develops from the three apical cells of the former. These three cells and one or two more cells adjacent to them come entirely from the apical cell of the two-celled proembryo. The further differentiation of these three cells into different parts of the mature embryo may be stated as follows. The apical cell forms the stem tip and the two cotyledons. The penultimate cell forms the hypocotyl and the greater part of the radicle. The third cell from the apex completes the apex of the radicle and, therefore, is the hypophysis.

This naturally takes us to the details about the further development of these different cells. The apical cell is the first to divide in a longitudinal manner (Figs. 38, 52, 53 and 66). Another longitudinal wall at right angles to the first appears in the same cell and four cells are formed (Figs. 42 and 55). All of them divide now transversely to form an octant (Figs. 43, 44, 57 and 58). In the meanwhile, the penultimate cell also divides longitudinally (Figs. 39 to 43 ; 54 to 58 and 67). The plane of this division generally is at right angles to that of the first longitudinal division in the apical cell. Along with the octant formation in the apical cell the penultimate cell also divides once more and four cells are formed. The sequence about the appearance of longitudinal divisions is, therefore, from the apical towards the micropylar cells. One important fact emerges out of this. Within the Centrospermales a group of families can be set apart, where the first longitudinal division appears in the apical cell and then it progresses towards the micropylar end. Illustrations of this type are found in the Nyctaginaceæ (Kajale, 1938), and the Phytolacaceæ (Kajale, unpublished). Some indications of the occurrence of similar sequence of longitudinal divisions in the Molluginaceæ are seen in the figures of Bhargava (1934, text-figures 5 and 6). In another group of families longitudinal division in the proembryo starts either in the second, third, fourth or even fifth cell from the apex and then it gradually extends towards the apical cell of the proembryo. This sequence is just the reverse of what is found in the first group of families, and its examples are found in the Chenopodiaceæ (Souèges, 1920), Caryophyllaceæ (Souèges, 1924) and Amarantaceæ (Joshi and Kajale, 1937).

After the longitudinal walls have been completed in the apical and penultimate cells, periclinal divisions begin to appear (Figs. 44 and 68). This is the beginning of the dermatogen differentiation. It begins in the tier formed by the penultimate cell and is later completed in the apical tiers (Figs. 44, 46, 68 to 71). It should be noted here, that the basipetal differentiation of the dermatogen appears distinctly characteristic of the Centrospermales and no deviation from this rule has yet been observed in any member of this order.

After the completion of the dermatogen, the divisions in the tiers formed by the apical and penultimate cells are not equal. The tier resulting from the latter cell divides more actively than the other

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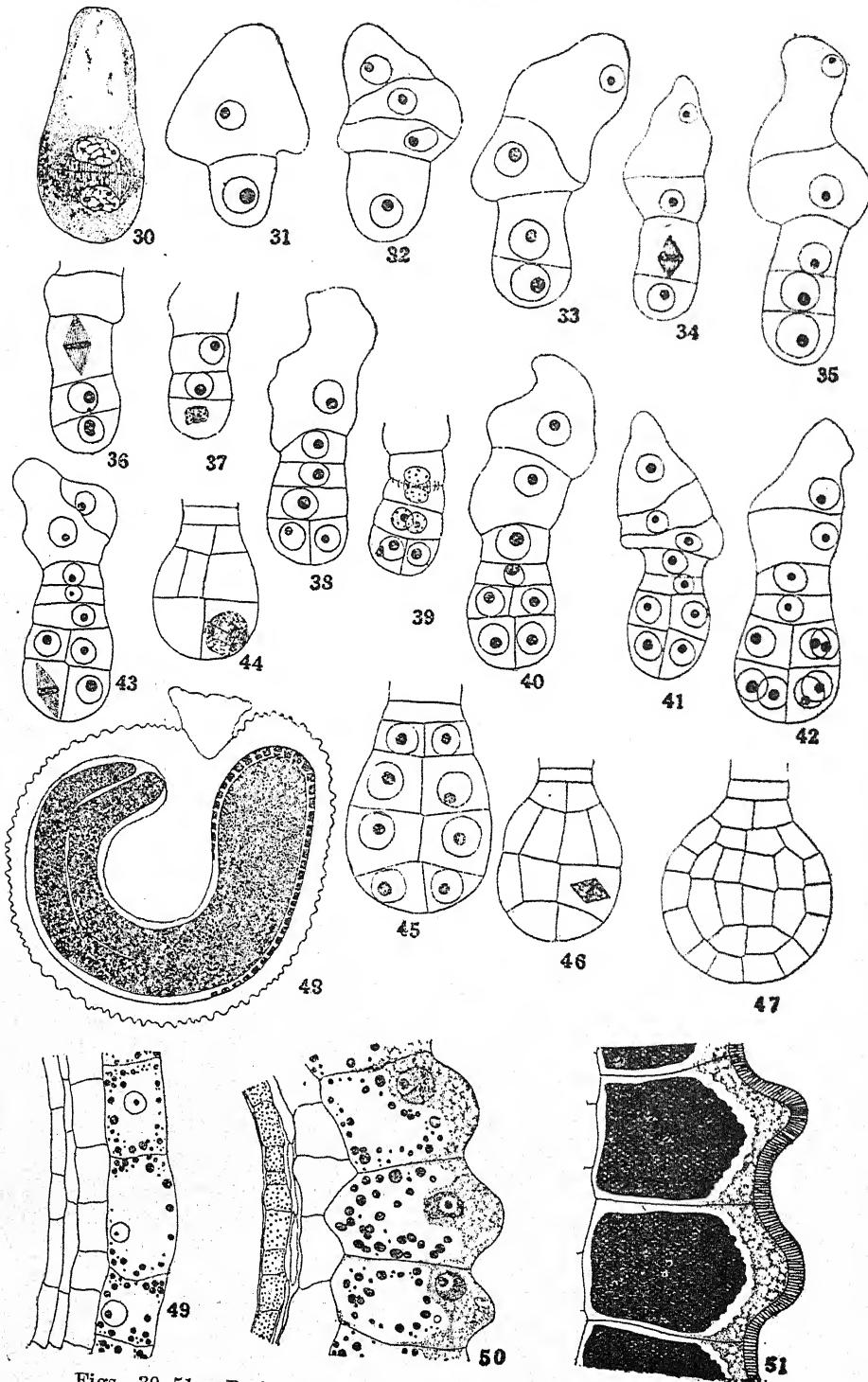
Figs. 30-51. *Portulaca quadrifida.* Figs. 30-47. Various stages in
the development of embryo. In Figs. 44-47 suspensor is not shown.
Fig. 48. A longitudinal section of the mature seed showing the embryo,

both by transverse and longitudinal walls, and forms the hypo-
cotyl and the greater part of the radicle.   As seen in the longitudinal
section at about the stage when the cotyledons appear (Fig. 73),
one or two peripheral layers below the dermatogen function as the
periblem, while the central core enclosed by the latter forms the
plerome.
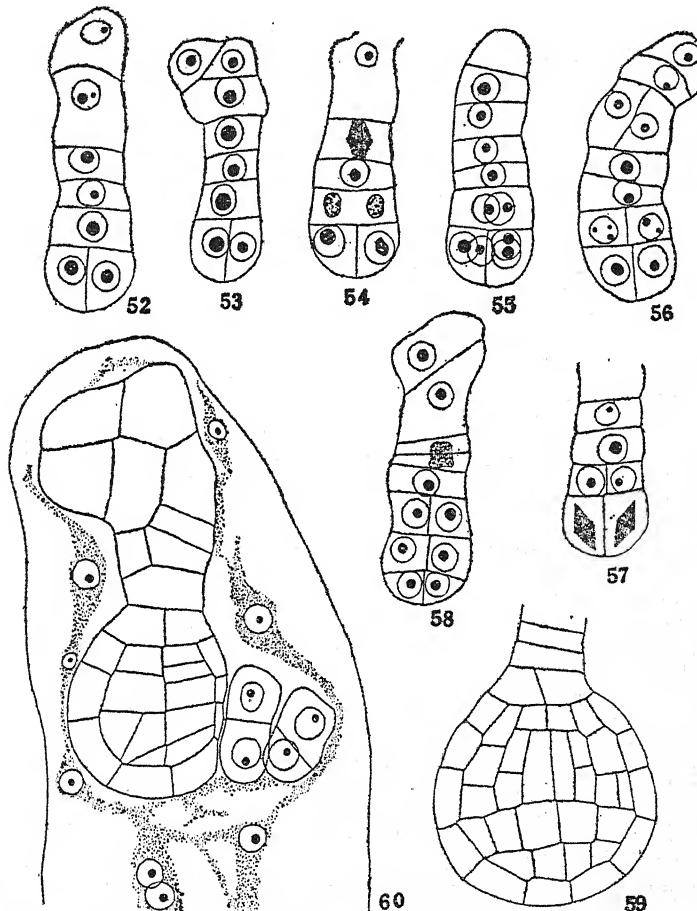
Figs. 52–60.   *Portulaca oleracea*.   Figs. 52–59.   Various stages in the
development of the embryo.   In Fig. 59 suspensor is not shown.   Fig. 60.
Micropylar part of the embryo-sac showing three embryos developing side.
by side (× 450).

The cells of the two tiers resulting from the apical cell divide
chiefly by longitudinal walls (Figs. 72 and 73).   After the embryo has
reached the size shown by Fig. 73 the central part develops into the

perisperm and a layer of endosperm.   Figs. 49–51.   Various stages in the
development of testa.   (Figs. 30–47 × 450; Fig. 48 × 50; Fig. 49
 × 450;   Figs. 50–51 × 250.)

stem tip, while the two cotyledons are formed from the peripheral region. Once the cotyledonary initials are formed their growth is very active and two large cotyledons are formed (Figs. 48 and 74).

The third cell from the apex deserves consideration now. This cell functions directly as the hypophysis. It first divides by two longitudinal walls forming four cells simultaneously with the differentiation of dermatogen in the apical tiers (Figs. 45, 46, 58 and 68). These cells do not divide further till rather a late stage of embryo development. Ultimately they complete the apical part of the radicle including the root cap.

Excluding the three apical cells of the procmbryo the remaining cells develop into the suspensor. It should be noted that the suspensor does not exclusively develop from the micropylar cell of the two-celled proembryo, but is also formed by a few cells derived from the apical cell. The structure of the suspensor differs in the three species of *Portulaca*. In *P. quadrifida* it consists of three to five cells only. It does not become massive in any part and generally remains uniseriate till it degenerates (Figs. 41 and 43). In the other two species it is more massive particularly at the micropylar end (Figs. 53, 68–70 and 73). Towards the embryo it is mostly uniseriate. This will be clear from Figs. 53 and 68–73. In *P. oleracea* it is observed that the cells of the suspensor sometimes divide by longitudinal walls quite at an early stage (Fig. 53). In all the three species no trace of the suspensor is seen in the mature embryo.

MATURE EMBRYO

The mature embryo is annular as in the Centrospermales in general (Figs. 48 and 74). It encloses within itself a large part of the nucellus that persists in the mature seed as perisperm. Abundant starch grains are deposited in the embryo. Roughly speaking these are uniformly distributed in the different parts of the embryo, except in the apical part of the radicle, where comparatively less starch is deposited. The size of the starch grains is also smaller in this part. In the region of plerome again the starch grains are smaller in size than in the other histogenic layers.

POLYEMBRYONY

A case of polyembryony has been observed in *Portulaca oleracea*. It is sketched in Fig. 60. There are three embryos developing side by side in the same embryo-sac. All these embryos are not developed to the same extent. One of them is much bigger than the other two. In the bigger embryo the dermatogen formation has been completed and the presence of suspensor, which is massive in the micropylar part, is also clearly seen. The other two embryos have not developed so much. Each one of them consists of two cells only. It follows from this that they have either developed much later than the bigger embryo, or their development has been slow.

The bigger embryo appears to have developed in the usual manner from a normal fertilised egg. The origin of the other two embryos is not very clear. Perhaps they have developed from

endosperm nuclei. At a stage represented by the bigger embryo in other embryo-sacs the structures other than the egg and endosperm nuclei, *i.e.*, the synergids and the antipodals, all disappear.
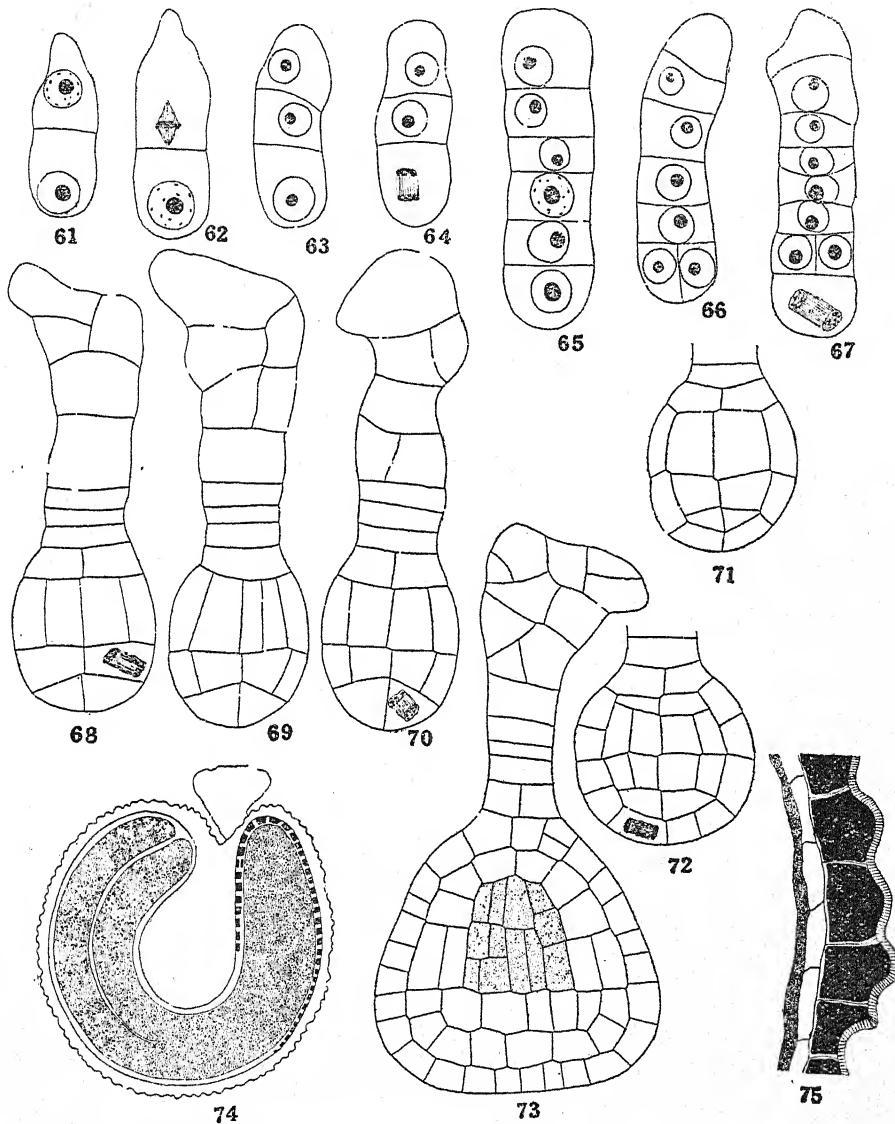
Figs. 61–75. *Portulaca grandiflora.* Figs. 61–73. Various stages in the development of the embryo. In Figs. 71 and 72 suspensor is not shown. Dotted part in Fig. 73 represents the plerome. Fig. 74. Longitudinal section of the mature seed showing embryo, perisperm and a layer of endosperm. Fig. 75. A part of the testa as seen in longitudinal section. (Figs. 61–72 × 450; Figs. 73 and 74 × 400; Fig. 75 × 250.)

It is, therefore, unlikely in the present instance that these embryos could have developed either from the synergids or the antipodals. Likewise they could not have developed from the nucellus cells since they are situated well within the embryo sac. They are not connected with the cells of the nucellus. Their origin appears to be from two endosperm nuclei. Authentic cases of the development of embryos from endosperm cells are very rare. As a matter of fact previously no example is known where an embryo has actually been seen developing from endosperm that had been produced as a result of triple fusion. Jeffrey and Haertl (1939) found in *Trillium* that embryos are derived from the endosperm nucleus, but in this case neither the egg nor the endosperm nucleus is fertilised. Two geneticists, Yamamoto (1936) and Kostoff (1939), however, have suggested that triploids in wheat and rye respectively develop from the endosperm, since in polyembryonous seeds they occur more frequently than haploids in conjunction with diploids.

PERISPERM AND ENDOSPERM

The central part of the nucellus persists in the mature seed as perisperm. This is enclosed by the embryo. It is a pear-shaped body with cells full of starch grains.

The endosperm formation begins at first in a free nuclear fashion. Later the endosperm becomes cellular. The wall formation starts at the micropylar end of the embryo-sac as observed by Joshi and Kajale (1937) in *Alternanthera sessilis* and *Digera arvensis*. It then extends towards the chalazal extremity and the endosperm completely becomes cellular. In this respect my observations differ from those of Rócen (1927). He says that in *Portulaca oleracea* the endosperm becomes cellular only in the micropylar part of the embryo-sac. In the slides I have examined the endosperm in all the three species of *Portulaca* becomes completely cellular during the development of the embryo. The greater part of it is absorbed by the growing embryo and in the mature seed it is represented by a layer or two surrounding the radicle in a cap-like fashion. In these persisting cells a large number of starch grains are deposited (Figs. 48 and 74).

TESTA

The early stages in the development of the testa have been studied only in *Portulaca quadrifida*, while the later stages have been studied in all the three species described in the paper. It has been shown previously that the integuments consist mostly of two layers of cells throughout their length (Fig. 49). Out of these four layers only three layers persist in the mature seed to form the testa. Two outer layers belong to the outer integument, while the third layer belongs to inner integument and is the inner layer. The outer layer of the inner integument is destroyed during the course of development (Figs. 50 and 75).

In the three layers that persist in the seed, some small grains first begin to be deposited in the outermost layer (Fig. 49). The

deposition of these grains begins some time before fertilisation. Similar grains are also deposited in the innermost layer (Fig. 50). The grains deposited in this layer are small in the beginning. The deposition starts generally from the micropylar end and extends towards the chalazal region. As more grains accumulate they fuse to form bigger ones and ultimately the cells are completely filled with them in the mature testa. But before this happens a marked change takes place in the cells of the outermost layer. The shape of these cells in the beginning is shown in Fig. 49 and they are somewhat cubical. Their nucleus does not occupy any fixed position. During the development of the seed, as the grains accumulate, the outer walls of the cells begin to bulge out. The nucleus now shifts into these convex outgrowths and the protoplasm also for some time is present mostly towards this side of the cells (Fig. 50). Meanwhile the deposition of the grains continues and the cells become entirely filled with these grains. The outer convex wall of the cells becomes greatly thickened. When the cells become completely filled up with these grains, the latter fuse to form a homogeneous mass in the mature testa (Figs. 51 and 75). The presence of similar structure in the outer layer of the testa was observed in *Portulaca grandiflora* also (Fig. 75) and the cells during their development undergo a similar series of changes. In *Portulaca oleracea* the cells of the outermost layer do not show any such difference between the peripheral and the remaining part of the cells, but are completely filled with the homogeneous mass of grains. The middle layer of the testa consists of parenchymatous cells in which no deposition of grains takes place even in the mature seed (Fig. 75).

SUMMARY

The development of the anther, ovule, male and female gametophytes and fertilisation in *Portulaca quadrifida* and the development of embryo, endosperm and seed in *P. quadrifida*, *P. oleracea* and *P. grandiflora* has been studied.

The archesporium in an anther-lobe consists of a single row of cells. The primary sporogenous cells directly function as pollen-mother cells. The tapetal cells are of parietal origin and become bi-nucleate. The inner wall of the tapetal and endothecial cells undergoes granular cutinization. The pollen grains are spherical and possess thirty furrows arranged in a pentagonal fashion. The male gametes are definite cells. Starch is present abundantly in the pollen grains. They are shed at the three-nucleate stage.

The ovules are ana-campylotropous. An air-space between the two integuments is seen in the early stages near the chalaza. The epidermal cells of the nucellus just below the micropyle simply stretch out radially, while the surrounding cells undergo periclinal divisions. The primary archesporium consists of a single hypodermal cell. A parietal cell is cut off. The megaspore-mother cell forms a linear or sometimes a T-shaped tetrad. A normal 8-nucleate embryo-sac develops from the chalazal megaspore. The synergids

are hooked. The antipodals are three small cells. The polar nuclei do not fuse before fertilisation, but lie closely pressed against each other just below the egg. During fertilisation the two polar nuclei fuse simultaneously with a male gamete. The other male gamete fuses with the egg. The pollen tube swells on entering the embryo-sac and generally destroys one synergid during fertilisation.

The first division of the egg is by a transverse wall. The micropylar cell divides transversely to form up to three cells. After the first transverse division of the apical cell the resulting micropylar cell divides again transversely and its daughter cell towards the micropyle may behave again in the same manner. Thus a pro-embryo of five to seven cells is organized. Three apical cells form the embryo proper. The rest form the suspensor. The most apical cell forms the stem tip and two cotyledons. The penulti-mate cell forms the hypocotyl and the radicle. The third cell completes the root tip and root cap. The mature embryo is annular. Numerous starch grains are deposited in different parts of the embryo. The suspensor is uniseriate in *P. quadrifida*. In other two species it is multiseriate at the micropylar end.

The nuclear endosperm gradually becomes cellular throughout the embryo sac. In the mature seed it is represented by a layer or two surrounding the hypocotyl and the radicle. The central part of the nucellus persists in the mature seed as perisperm and contains abundant starch.

The testa of the mature seed consists of three layers of cells. In the outer and inner layers some kind of grains fill the cells completely. In the middle layer no such grains are deposited. In *P. quadrifida* and *P. grandiflora* the cells of the outermost layer of the testa prominently bulge out, and due to this the testa becomes warty.

I welcome this opportunity to acknowledge my grateful thanks to Dr. A. C. Joshi for his guidance and helpful criticism.

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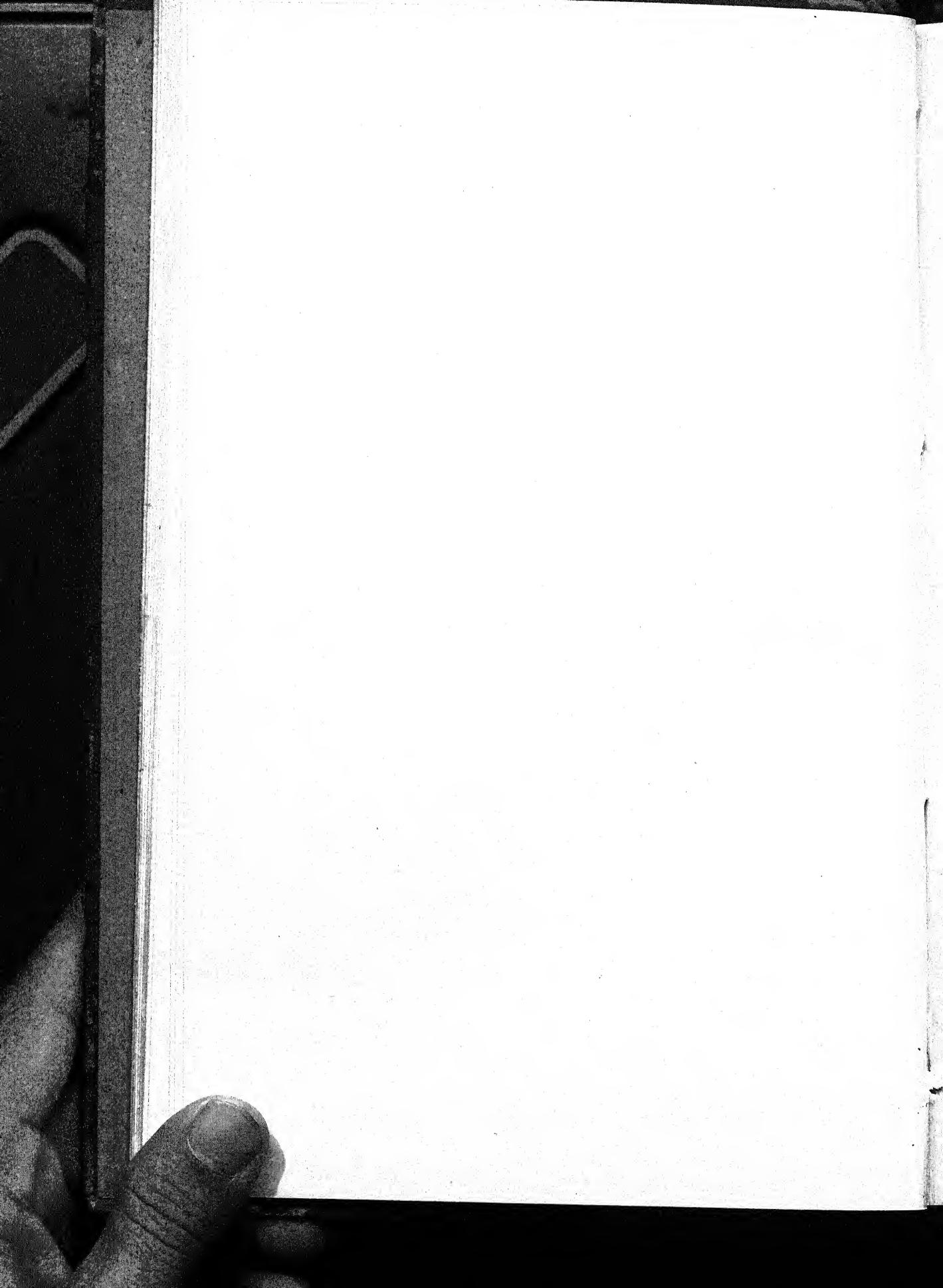
STUDIES IN OXALIDACEÆ

(*Biophytum sensitivum*, DC., *Averrhoa Carambola*, L., and *Averrhoa Bilimbi*, L.)

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(Communicated by C. V. Krishna Iyengar)

Of the seven genera of Oxalidaceæ, *Oxalis* and *Biophytum* are the only two studied by previous workers. *Oxalis* engaged their attention earlier and serveral species of that genus have since been investigated. Jönsson (1880) reported a normal type of megaspore development in *Oxalis Acetosella*. In the same plant, Samuelsson (1913) observed a free nuclear endosperm. Schürhoff (1924) dealing with several species of *Oxalis*, confirms Samuelsson's observation regarding the endosperm formation in *Oxalis Acetosella*. No haustorial structures are associated with the endosperm, but he finds in that member, a suspensor haustorium, with enlarged and deeply staining nuclei. He mentions that the haustorial activity is brief and that the haustorium can be recognized only with difficulty during later stages. Accounts vary regarding the suspensor haustorium in the other species of *Oxalis*. While Billings (1901) reports the absence of a suspensor haustorium in *Oxalis valdiviensis*, Hammond (1908) notices in *Oxalis corniculata* that the integument is pierced by a haustorium of suspensorial origin. Comparing the suspensor haustoria in *O. Acetosella* and in *O. stricta*, Schürhoff mentions that the haustorium of the latter is smaller in size and has a briefer activity than in the former.

In his account of microsporogenesis in several species of *Oxalis*, Schürhoff (1924) reports the absence of a tapetal periplasmodium and the presence of three-nucleate pollen grains at the time of shedding. The haploid number of chromosomes in *Oxalis floribunda* is given as 7. In his study of Gruinales, Mauritzon (1934) included two species of *Biophytum*. Noll (1935) restricts himself to the development of the embryo in *Biophytum dendroides*, DC. It is seen however, that in most of the accounts mentioned above, greater attention has been paid towards embryogeny than to the development of the embryo-sac. Mauritzon reports a normal type of embryo-sac in *B. sensitivum*. But my observations indicate that the development of the embryo-sac in this plant is of the *Allium-type*.

Material and Methods

The material of *Biophytum sensitivum* was collected from the Ayurvedic Gardens, Mysore. Flowers and fruits of *Averrhoa*

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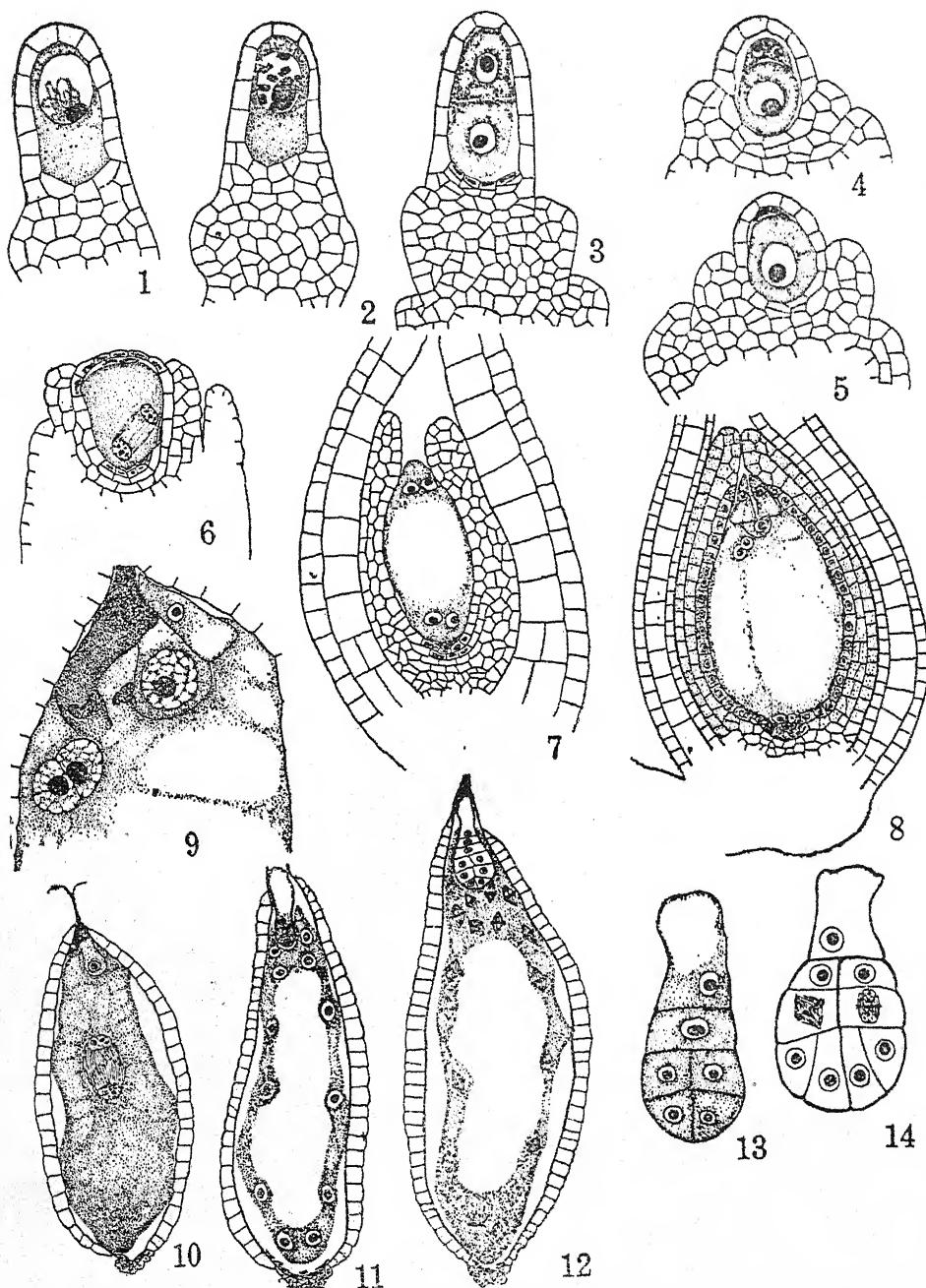
Figs. 1–14.—*Biophytum sensitivum*. Fig. 1. Megaspore mother cell (× 640). Fig. 2. Megaspore mother cell in Diakinesis (× 640). Fig. 3. Dyad stage (× 730). Fig. 4. Division of the upper dyad cell

Carambola and *A. Bilimbi*, in various stages of development were collected from the gardens at Krishnarajasagara. Bouin's Fluid and Acetic Alcohol with 30% of glacial Acetic acid were used as fixatives and gave fair results. More satisfactory results were obtained by fixation in Nawaschin's fluid. In the case of *Averrhoa* it was found necessary to fix the ovules separately as the wall of the berry is very fleshy. Sections varied in thickness from 8 to 16 microns and were stained in Heidenhain's Iron-alum Hæmatoxylin.

MICROSPOROGENESIS

In the young anther a row of hypodermal cells, easily distinguished by their deeper stain and prominent nuclei, is differentiated as the primary archesporium (Fig. 35). In all the three plants the primary archesporial layer divides periclinally to cut off a primary wall layer on the outside. This divides further to form three to four wall layers between the epidermis and the sporogenous tissue. The outermost of these wall layers, the endothecium, enlarges and its cells soon develop the characteristic fibrous thickenings (Figs. 36–37). The innermost layer which is in contact with the sporogenous cells forms the tapetum. The tapetal cells are much enlarged and stain very deeply on account of their rich contents. Their nuclei also enlarge and divide so that during the later stages each tapetal cell has 2 to 4 nuclei. The tapetum seems to be most active at the time of division of the microspore mother cells and disintegrates soon after. A tapetal periplasmodium was not noticed in any instance. By the enlargement of the endothecium and tapetum the middle layers are crushed at an early stage.

The sporogenous cells divide to form the microspore mother cells which round off from their neighbours and undergo the usual prophasic stages preceding meiosis. The pairing of chromosomes is very clearly seen in the pachytene stage (Fig. 38) and the pairs of chromosomes could be easily counted at diakinesis (Fig. 39). In *Biophytum* the distinctness of the chromosomes was retained even during the interphase (Fig. 41).

Chromosome counts were made at diakinesis and from polar view of the metaphase plates during the first and second divisions. In *B. sensitivum* the haploid number of chromosomes is ten. In *Averrhoa Bilimbi* it is eleven and in *Averrhoa Carambola* it is twelve. In all the three plants the pollen grains are bi-nucleate (Figs. 42–46) at the time of shedding. In *Biophytum*, the pollen grain is elongated

the lower dyad cell enlarging ($\times$ 480). Fig. 5. Degeneration of the daughter cells of the upper dyad cell ($\times$ 480). Fig. 6. Two-nucleate embryo-sac, nucellus shows signs of disintegration ($\times$ 480). Fig. 7. Four-nucleate embryo-sac, nucellus completely disappeared ($\times$ 365). Fig. 8. Eight-nucleate embryo-sac ($\times$ 262·5). Fig. 9. Micropylar portion of the embryo-sac showing fertilisation. ($\times$ 730). Fig. 10. First division of the primary endosperm nucleus ($\times$ 262·5). Figs. 11–12. Development of endosperm ($\times$ 160). Figs. 13–14. Stages in development of embryo (Figs. 13 and 14, $\times$ 730).

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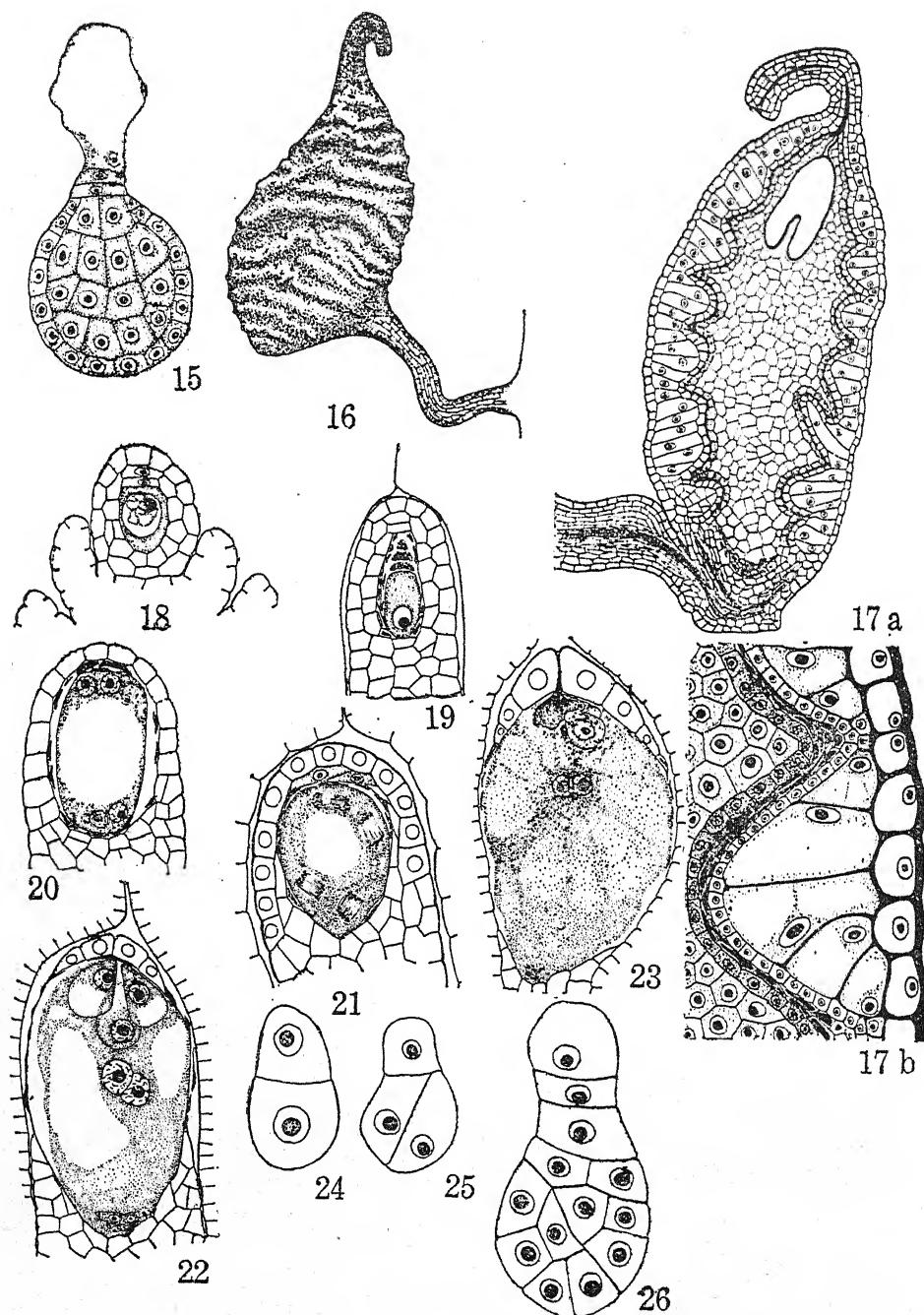
Figs. 15–26.—*Biophytum sensitivum.* Fig. 15. Stages in development of embryo (× 525). Fig. 16. A fully formed seed (×　). Fig. 17a. L.S. of a seed (× 45). Fig. 17b. A portion of the wall of the seed

while in the other two plants it is spherical and all the three are of the tricolpate type (Wodehouse, 1935).

OVULE

In *Biophytum sensitivum* as well as in the two species of *Averrhoa* the nucellus arises as an outgrowth on the axile placental ridge and by the multiplication of the cells at its base, is soon borne at the tip of a long stalk—the funiculus. While the nucellus in *Biophytum* is reduced and presents a conical appearance, in *Averrhoa* it is more massive.

The inner integument appears first as a ring of cells at the base of the nucellus and soon envelops it, leaving a micropyle at the top. The outer integument develops slightly later but very soon outgrows the inner. In the case of *Biophytum* the tip of the outer integument is prolonged as a curved and tubular structure whose significance in the dehiscence of the fruit and the dispersal of the seed is as in *Oxalis*. The inner integument in all the three plants has three layers of cells the innermost of which develops into a deeply staining, epithelial layer surrounding the endosperm. The two outer layers are very much compressed by the increase in thickness of the outer integument and only their remains can be seen in a mature seed (Fig. 17*b*).

In *Biophytum* the outer integument presents some interesting features as noticed by Mauritzon. Of the three layers of cells comprising it, the middle one enlarges very much and as some of its cells enlarge more than the others, the layer appears wavy in section (Fig. 17*a*) and the cells have their outer tangential walls thickened. In the outer layer also the outer tangential wall is very much thickened. The inner layer of the outer integument does not undergo any conspicuous change and is seen against the compressed and degenerating cells of the outer layer of the inner integument (Fig. 17*b*).

MEGASPOROGENESIS

In all the three plants, the primary archesporium is represented by a single hypodermal cell which enlarges and is conspicuous on account of its rich contents. A multiple archesporium has not been met with, in any of the plants (Figs. 1, 18 and 27).

In the two species of *Averrhoa* the primary archesporial cell cuts off a primary parietal cell which divides periclinally or slightly obliquely, to give rise to two parietal cells. They degenerate during later stages but their remnants can be made out above the embryo-sac even in the four-nucleate condition (Figs. 20 and 29). The

enlarged (× 365). *Averrhoa Bilimbi.* Fig. 18. Megaspore mother cell with two parietal cells above (× 320). Fig. 19. Linear tetrad showing degeneration of the upper three megaspores (× 480). Fig. 20. Four-nucleate embryo-sac (× 480). Fig. 21. Eight-nucleate embryo-sac (× 525). Fig. 22. Mature embryo-sac (× 480). Fig. 23. Embryo-sac at the time of fertilisation (× 525). Figs. 24–26. Stages in development of embryo (Figs. 25 and 26 × 730).

megaspore mother cell in *Averrhoa Carambola* and also *Averrhoa Bilimbi*, develops further and undergoes two divisions to give rise to a linear tetrad of megaspores. Of these, the upper three degenerate while the chalazal functions to give rise to the embryo-sac (Figs. 19 and 28).

In *Biophytum sensitivum* the condition is different. Here the primary archesporial cell functions directly as the megaspore mother cell without cutting off any parietal tissue. The nuclear changes preceding the first division of the cell, are meiotic and the number of chromosome pairs at the diakinesis stage is ten, thus confirming the count obtained in microsporogenesis. By the division of the megaspore mother cell two cells are formed one placed above the other (Figs. 1–3). The upper of these two dyads is often seen to be slightly smaller than the lower and undergoes a longitudinal division so that at this stage (Fig. 4) there are two small cells side by side above the distinctly larger lower dyad cell. The former soon degenerate and the latter undergoes further development to give rise to the embryo-sac so that the development conforms to the *Allium*-type. As the division in the upper dyad cell is vertical resulting in three cells arranged in the form of a T, the difficulty of interpreting the row of three cells as mentioned by Maheshwari (1937) does not arise in the present case. In *Biophytum* degeneration sets in in the two cells formed by the division of the upper dyad but it is said to start earlier, *i.e.*, even before the completion of the division—in *Lycopsis* by Svensson (1925). Cases have also been reported as in *Scilla* (Hoare, 1934), where the lower dyad disintegrates while the upper develops into the embryo-sac.

The nucellar epidermis shows signs of very early degeneration in *Biophytum*, whereas in *Averrhoa*, the nucellus persists for a considerable time and can be clearly seen above the developing embryo-sac. In both the species of *Averrhoa*, two cells of the nucellar epidermis situated below the micropyle are seen to be more prominently developed than the rest and their nuclei are also larger (Figs. 23 and 29).

At the base of the enlarging embryo-sac, a few cells show degneration due probably to the activity of the embryo-sac (Fig. 7).

EMBRYO-SAC

The mature embryo-sac is eight-nucleate in all the three plants. The two synergids are pearshaped and vacuolate at their enlarged posterior end. The two polar nuclei lie in the vicinity of the egg and fuse only at the time of fertilisation. The three antipodals are small and degenerate very soon after fertilisation. In *Biophytum* they are situated in a small pit at the base of the embryo-sac (Fig. 8).

FERTILISATION

Double fertilisation has been observed in *Biophytum sensitivum* and *Averrhoa Bilimbi*. At the time of fusion of the male nucleus with the egg, the nucleus of the latter is seen to be in the resting

stage (Fig. 9). During its entry into the sac the pollen tube destroys one of the synergids and the other degenerates soon afterwards.

ENDOSPERM

The Endosperm in all the three plants develops by free-nuclear division. The primary endosperm nucleus migrates to the central part of the embryo-sac where the first division occurs. In the four-nucleate stage of the endosperm, one of the endosperm nuclei is near the egg, one is situated at the chalazal end and the other two are lateral in position. By the continued divisions of these nuclei, the peripheral layer of cytoplasm of the embryo-sac becomes studded by a number of nuclei (Figs. 10–12). Wall formation begins only after the number of nuclei in the endosperm has exceeded thirty-two and proceeds from the periphery towards the centre of the embryo-sac. In *Biophytum* the periphery of the endosperm is wavy in outline due to unequal enlargement of the cells of the outer integument, as previously mentioned.

EMBRYO

In *Biophytum*, as well as in the two species of *Averrhoa*, the fertilised egg rests for a considerable period before undergoing a division. The first division is transverse in all the three plants. By the divisions of the primary suspensor cell thus separated, a suspensor is formed with its basal cell considerably larger than the other cells. Since its contents do not take up a deep stain, and the cells of the surrounding region do not appear to be affected by it in any way, a haustorial activity cannot be ascribed to it. In the species of *Averrhoa* the basal cell is even less conspicuous than in *Biophytum*.

The further development of the embryo follows different lines in the two genera. In *Biophytum* the division in the embryonal cell is vertical. This is followed by the usual quadrant and octant stages and the further development is as described by Mauritzon in this plant. The embryogeny of *B. dendroides*, DC. has been studied in very great detail by Noll, and it is seen that the development of embryo in the two species is similar, and resembles the development of the embryo in *Urtica pilulifera* (Souèges, 1921) (Figs. 13–15).

In the two species of *Averrhoa*, the first division in the primary embryonal cell is followed by an oblique wall (Fig. 25). The next division is also oblique and meets the previous wall at an angle (Fig. 32). Further development takes place by divisions followed by transverse as well as oblique and intersecting walls. The three primordial layers are soon established. The development of the embryo in *Averrhoa* is thus seen to follow the *Geum-type* reported by Souéges (Figs. 24–26 and 31–34).
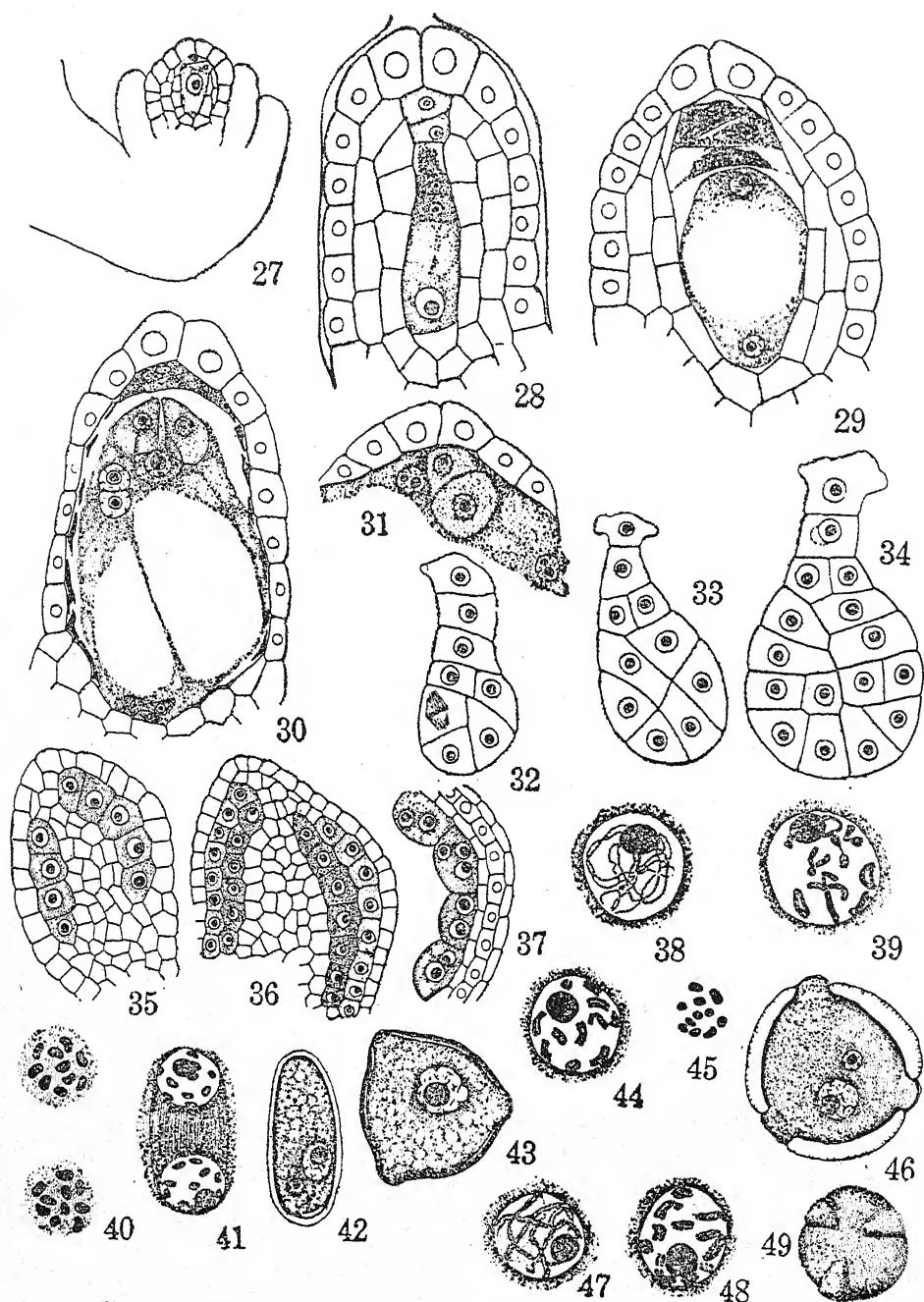
Figs. 27–49.—*Averrhoa Carambola*. Fig. 27. Megaspore mother cell with two parietal cells (× 365). Fig. 28. Linear tetrad, note the enlargement of the two apical cells of the nucellus (× 1095). Fig. 29. Two-nucleate embryo-sac (× 1095). Fig. 30. Eight-nucleate embryo-sac

DISCUSSION

A review of available literature on the Oxalidaceæ, reveals that more attention has hitherto been bestowed on the study of embryogeny than on the development of the gametophytes. A suspensor haustorium varying in size and activity has been reported by several authors in *Oxalis*. The development of the embryo-sac has been reported to follow normal lines.

In 1934 Mauritzon included two species of *Biophytum* in his embryological studies on Gruinales. Noll (1935) confined himself to a detailed study of the embryogeny in *B. dendroides* and does not mention anything about the development of the embryo-sac.

Mauritzon reports a normal type of embryo-sac in *Biophytum sensitivum*. This conclusion is based according to his own statement on the study of a poorly fixed preparation. Only a single figure of the embryo-sac has been drawn showing an egg, a synergid and the secondary nucleus. In the present investigation the series of stages obtained in the development of the embryo-sac show that the development is not normal as hitherto reported but of the *Allium-type*. No mention is made by Mauritzon of the presence of parietal cells in *Biophytum*. The same author reports parietal cells in *Erythroxylum* while they are absent in *Radiola*.

Among the dicotyledonous families allied to Oxalidaceæ an *Allium-type* of embryo-sac is reported in *Xanthoxylum alatum* and *X. Bungei* of Rutaceæ by Mauritzon (1935). In Malpighiaceæ, Stenar (1937) noted an *Allium-type* of embryo-sac in *Galphimia gracilis* and Subba Rao (1939) in *Malpighia glauca*. In *Impatiens Sultani* Ottley (1918) reported an *Allium-type* but Schürhoff (1924) believes it to be of the *Normal-type*. It must however be mentioned that the *Allium-type* of embryo-sac does not offer any clue regarding the inter-relationships of the families or even the genera, as it has been reported in several families (Maheshwari, 1937) only a few of which are closely related. In the Oxalidaceæ itself only *Biophytum sensitivum* shows an embryo-sac of the *Allium-type*, the others being normal.

The development of the embryo is found to be different in the two genera. In *Biophytum* the embryogeny is of the *Urtica pilulifera-type* (Mauritzon and Noll), while in *Averrhoa* sp. it is similar to *Geum* (Souèges, 1921).

(× 730). Figs. 31–34. Stages in development of embryo (× 730). *Biophytum sensitivum.* Figs. 35–37. Stages in development of the anther and the tapetum (× 525). Fig. 38. Pachytene in the microspore mother cell (× 1460). Fig. 39. Diakinesis (early) (× 1460). Fig. 40. Polar view of the metaphase plates at the second division (× 2920). Fig. 41. Interphase (× 1460). Fig. 42. Pollen grain in long. section (× 730). Fig. 43. Pollen grain in tr. section (× 1460). *Averrhoa Bilimbi.* Fig. 44. Diakinesis in the microspore mother cell (× 1460). Fig. 45. Polar view of the metaphase plate during first division (× 1460). Fig. 46. Pollen grain in tr. section (× 1460). *Averrhoa Carambola.* Fig. 47. Pachytene in the pollen mother cell (× 1460). Fig. 48. Diakinesis (× 1460). Fig. 49. Pollen grain (× 730).

SUMMARY

Microsporogenesis proceeds normally in all the three plants. The tapetal cells become two- or four-nucleate. The haploid numbers of chromosomes in *Biophytum sensitivum*, *Averrhoa Bilimbi* and *Averrhoa Carambola*, are ten, eleven and twelve respectively. The pollen grains in all the three plants are tricolpate and contain two nuclei.

A single hypodermal cell represents the primary archesporium in all the three plants.

In *Averrhoa Carambola* and *Averrhoa Bilimbi*, two parietal cells are formed. The megaspore mother cell gives rise to a linear tetrad of megaspores, the lowest of which develops into the embryosac.

In *Biophytum* the archesporial cell directly functions as the megaspore mother cell. The upper dyad cell undergoes a longitudinal division but the daughter cells soon disintegrate. The lower dyad cell develops into the embryo-sac which is thus of the *Allium-type*.

The mature embryo-sac is eight-nucleate in all the three plants. The synergids are pyriform. The polar nuclei fuse at the time of fertilisation. The antipodals are small and degenerate soon after fertilisation.

The endosperm is free-nuclear in all the members.

The development of the embryo in *Biophytum* is of *Urtica pilulifera-type*, while in the two species of *Averrhoa*, it resembles that of *Geum* (Souèges, 1921).

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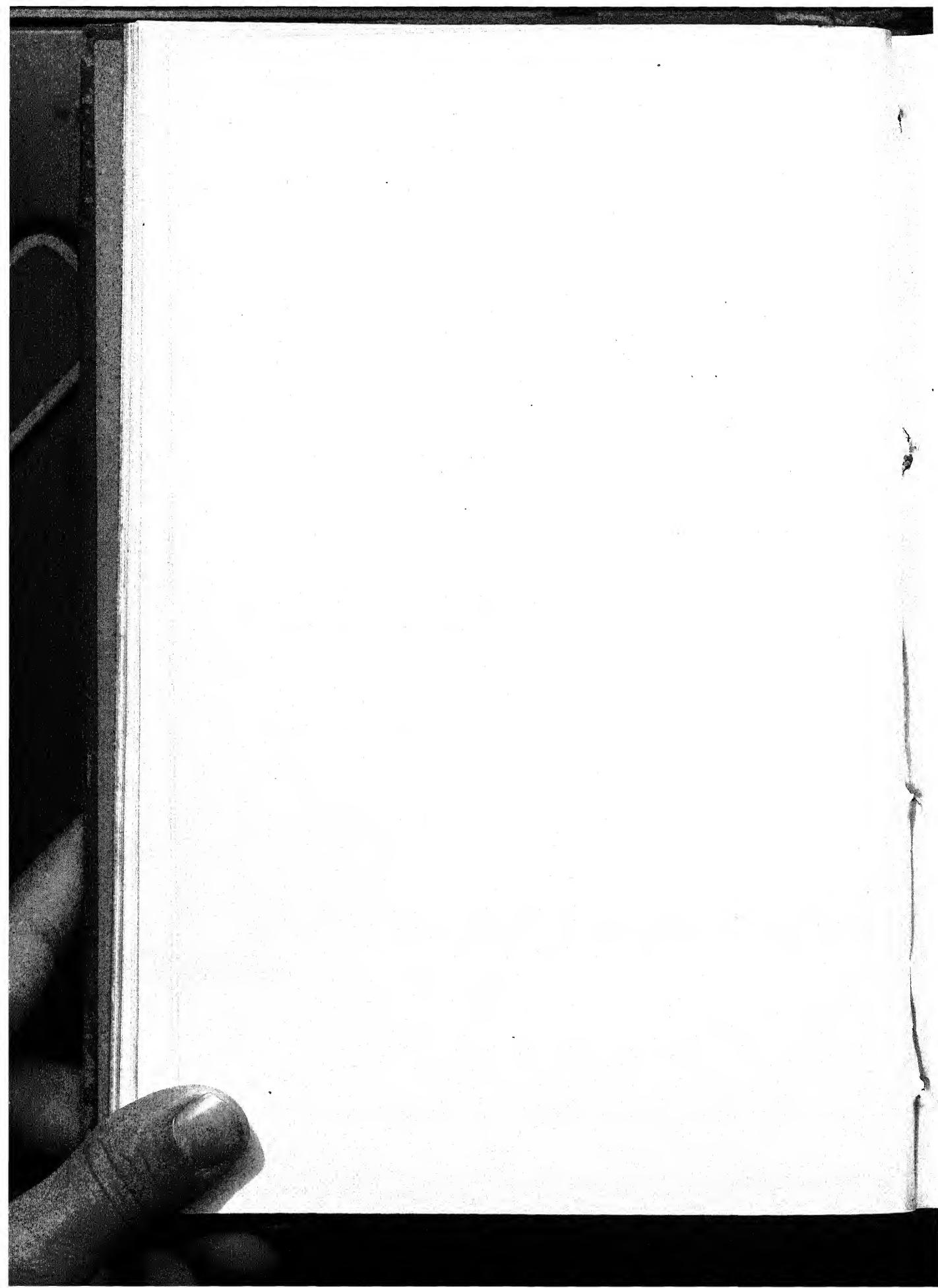
IMPORTANCE OF ANATOMICAL CHARACTERS OF THE SPOROPHORES IN THE TAXONOMY-STUDY OF THELEPHORACEÆ OF BENGAL

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THE delimitation of species in fungi till recently was based on external characters alone often leading to a large increase in their number particularly in families whose members are susceptible to variation in response to a changing environment. Attempts have, therefore, been made to utilise our increased knowledge of microscopical characters of fructifications for this purpose. The present tendency is, however, to use both the macroscopical and microscopical characters for defining species. The writer considers this to be a very sound procedure and hopes that its adoption will lead to a more rational and natural classification of the higher fungi.

Much stress has been laid on microscopical characters by recent workers, such as Burt[5] in his monograph on the *Thelephoraceæ*, Kauffman[9] in the *Agaricaceæ*, Patouillard[13] and Bourdot and Galzin[4] in the *Hymenomycetes* in general. Donk[8] revised the whole group of *homo-* and *heterobasidiomycetes* of Holland mainly on the basis of detailed anatomical studies. Pilat'[ ] in Europe and Bose[2,3] in Bengal are at present engaged in making extensive studies of the anatomical characters of *Polyporaceæ*. Overholts[12] in his 'Research methods in the taxanomy of Hymenomycetes' has shown how the microscopical structures are essential in the determination of a species of higher fungi. Mounce[11] has shown the importance of microscopic characters of the sporophores in culture as an aid in identifying some wood-destroying fungi. Corner[6,7] has made a detailed study of the hyphal systems composing the fruit-bodies of a few polypores and he hopes that a detailed study of the hyphal characters of the different species will give us the exact clue for natural grouping of higher fungi.

In the present paper the writer has put forward a detailed comparative account of the anatomical characters of the sporophores of Bengal species of Thelephoraceæ most of which have already been described by the writer.[1] For convenience, a comparative statement of the microscopical characters is given below. They have been described under the following heads :—

(1) Hyphal systems composing the fruit-bodies, and (2) conspicuous anatomical characters such as,—basidia, spores,

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cystidia, setæ, gleocystidia, paraphyses, hyphal pegs and conducting cells (lactiferous cells).

The methods followed in studying these anatomical characters have already been discussed by the writer in a previous paper.[1]

1. HYPHAL SYSTEMS

So far as the Bengal species are concerned three different systems of hyphæ, *viz.*, *skeletal.*, *generative* and *binding*, have been found involved in the construction of the fruit-bodies of the Thelephoraceæ. The writer has followed Corner[7] in using the terms *dimitic*, *i.e.*, composed of two systems of hyphæ, *trimitic*, has but three systems and *monomitic* with only one system of hyphæ.

Of the eighteen Bengal species of *Stereum*, the *trimitic* construction has been found in *S. nitidulum, S. vibrans, S. endocrocinum, S. hirsutum, S. petalodes, S. percome, S. elegans,* and *S. fuscum,* and it seems to be characteristic of the coriaceous species. The *dimitic* species are *S. scytale, S. umbrinum, S. fasciatum, S. crenatum, S. papyrinum, S. alternum, S. Schomburgkii, S. annosum* and *S. glabrescens.* Of the five species of *Hymenochœte* three are *dimitic* (*H. nigricans, H. aspera* and *H. tenuissima*) and the other two are of *trimitic* (*H. rubiginosa, H. cacao*) construction.

In the present paper, only the different systems of hyphæ that are involved in the construction, of the fruit-bodies of the Thelephoraceæ, have been recorded, but as to their development and inter-relationships the writer hopes to give a complete account later on. In characterising the different systems of hyphæ, the following characters have been taken into consideration :—Colour, width, wall-thickness, septation, branching, contents, clamp- and H-connections, and their disposition in the fruit-body.

2. CONSPICUOUS ANATOMICAL CHARACTERS

Basidia.—Basidia are the most important hymenial organs and they are packed close side by side to form the hymenium. They arise either from the prostrate hyphæ of the compact subiculum, *e.g.*, in the genus *Corticium* or from the erect hyphæ which before giving rise to the basidia form a sub-hymenial layer. Their shape varies between the two extreme types: the long basidium, narrowly clavate (*e.g.*, in *H. cacao*, Fig. 2) and short, broad and obovate basidium (*e.g.*, in *H. nigricans*, Fig. 1), each terminated by 2–4 spores on the sterigmata. Their dimensions, although varying within extensive limits, have been noted. Since the time of Fries (1874) the nature and the structure of the basidia and spores have been studied in great detail by numerous workers who have propounded from time to time several new schemes of classification and re-arrangement of species. Of recent years much stress has been laid on this character by Patouillard,[13] Rea[14] and Rogers.[15]

Spores.—Spores have always been found to be fairly constant for each species, in all the species so far studied, especially in colour, surface-marking, etc., and they serve as a valuable specific character.

Their shape varies within restricted limits; they are elliptical with an apiculus at the hilum and a little depressed at the anterior side (*e.g.*, in *S. fasciatum*, Fig. 5 and *S. hirsutum*) or oval (*e.g.*, in *S. percome*, Fig. 3), they become shorter and sub-globose (*e.g.*, in *S. scytale*, Fig. 4), sometimes becoming round (*e.g.*, in *H. aspera*, Fig. 6). Gutullets are sometimes present in certain species (*e.g.*, *S. percome*, Fig. 3). Their dimensions vary within wide limits, for instance, they are very large in the genus *Aleurodiscus*, but very small, about 1 to 2 μ in *Asterostromella rhodospora*.

Cystidia.—Cystidia on the whole are important specific characters, but sometimes they have been considered to be of generic import. The resupinate genera *Peniophora* and *Corticium* are separated from each other by the presence of these organs in the former and absence in the latter and thus their systematic value has been correspondingly increased. From the studies of the Bengal species of *Thelephoraceæ* made up to now it has been observed that they occur fairly commonly in the genus *Stereum*. They are in the main hymenial organs (*e.g.*, in *S. percome*, Fig. 7) but not infrequently they originate from the sub-hymenium (*e.g.*, in *S. umbrinum*, Fig. 8 and *S. Schomburgkii*). They project mostly to the level of the basidia (*e.g.*, in *S. umbrinum*) and occasionally they are embedded in the sub-hymenial region (*e.g.*, in *S. papyrinum*, Fig. 9). When extending beyond the basidial level they project upto 31 μ (*e.g.*, in *S. papyrinum*). Their shape is very variable being mostly conical (*e.g.*, in *S. papyrinum* and *S. percome*), sometimes becoming elongated, cylindrical, etc. Often they are heavily incrusted, partly (*e.g.*, in *S. percome*) or wholly (*e.g.*, *S. papyrinum*), the incrusting material being calcium oxalate in the main. They are colourless organs mostly but in the case of *S. umbrinum* they are brown in colour and according to Overholts this colour resides in the wall and not in the contents. In some species they are comparatively rare (*e.g.*, in *S. vibrans*) while in others they are very numerous (*e.g.*, in *S. papyrinum*).

Setæ.—Setæ have been considered both as generic and specific characters of Thelephoraceæ since the time of Léveillé and their importance is very great from systematic standpoint. Léveillé was the first who isolated the genus *Hymenochæte* from the genus *Stereum* on the presence of these organs which darken on the application of KOH solution.

Burt has laid much stress on the relative level at which setæ originate. Sometimes they originate from the base of the basidial layer (*e.g.*, in *H. aspera*, Fig. 11), while in others they originate from every part right through the underlying tissues (*e.g.*, in *H. rubiginosa*, Fig. 10). Their extent of projection is restricted to or sometimes beyond the basidial level (*e.g.*, in all the five species, *Hymenochæte*). The projecting part beyond the basidial layer varies from 9–18 μ (*e.g.*, in *H. cacao*, Fig. 14) to 38–60μ (*e.g.*, in *H. tenuissima*, Fig. 12). Imbedded setæ are quite common in
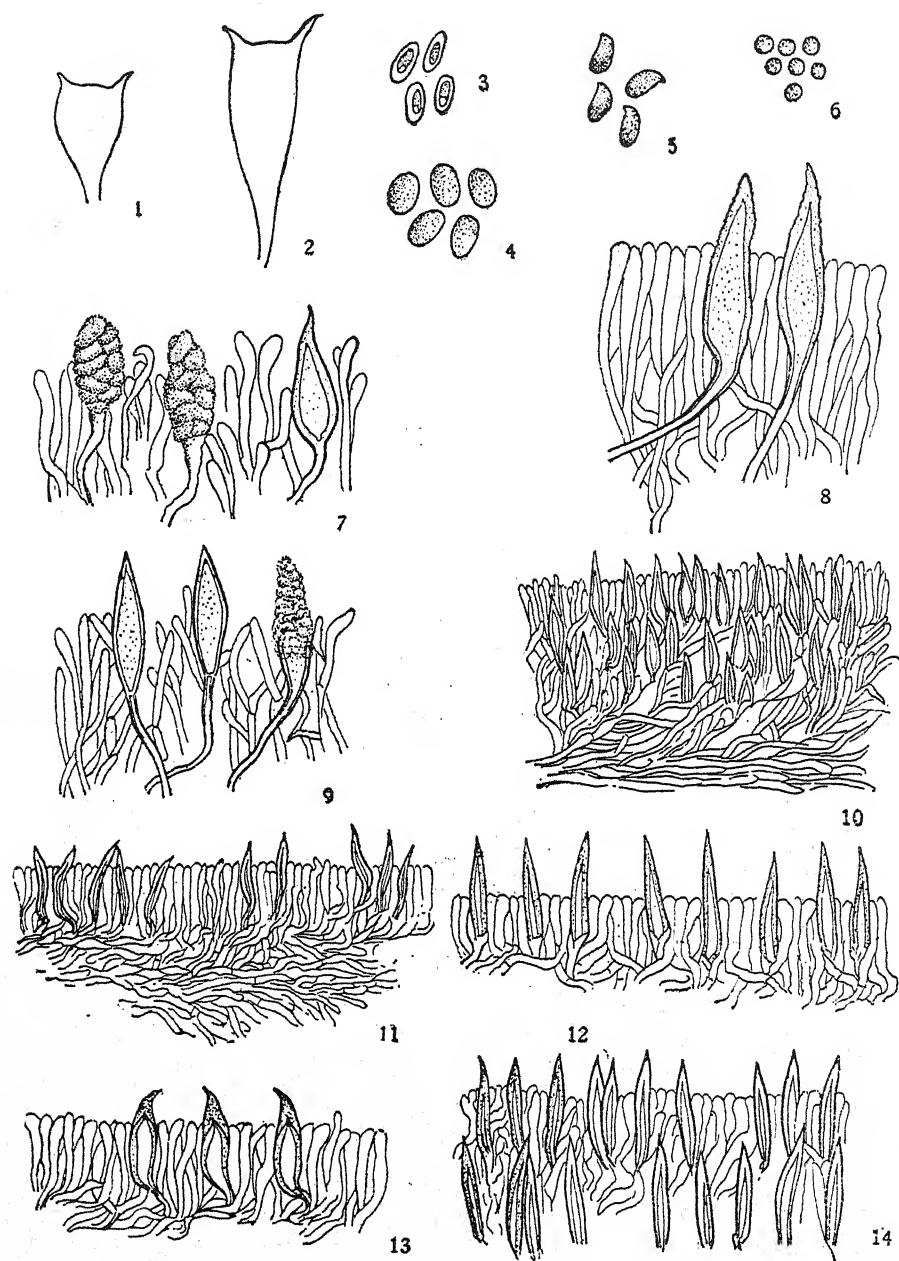
Figs. 1–14.—Fig. 1. Short, broad and obovate basidium of *H. nigricans*. Fig. 2. Long, narrowly clavate basidium of *H. cacao*. Figs. 3–6. Different types of spores. Fig. 7. Hymenial layer of *S. percome* showing conical and heavily incrusted cystidia. Fig. 8. Hymenial layer of

H. rubiginosa. Their comparative abundance has been considered to be of diagnostic value. Thus they may be few, frequent (*e.g.*, in *H. tenuissima*) or abundant (*e.g.*, in *H. rubiginosa*). Their size and form are quite variable. They are small about $18–31 \times 11 \cdot 5–6 \cdot 5 \mu$ (*e.g.*, in *H. cacao*) and attaining a dimension of $40–75 \times 8–10 \mu$ in *H. tenuissima*. They are generally broadly conical with a sharp point as in *H. rubiginosa* may often become cylindric with the apex narrowed to a point as in *H. nigricans*, Fig. 13). The tip character may be straight and sharp-pointed as in *H. aspera*, sometimes curved as in *H. nigricans*. They are always brown in colour.

Gleocystidia.—Gleocystidia sometimes form important specific characters by means of which many species of Thelephoraceæ can be conveniently separated. They are so rare in the genus *Stereum* that their presence in abundance forming a distinct compact layer in *S. fuscum* (Fig. 15) at once distinguishes it from other species. They are barely visible in *S. elegans* while in *S. nitidulum* (Fig. 16) they are quite common. These organs are always elongated and very frequently they are curved or flexuous in outline. They are hymenial organs (as in *Corticium*) but more frequently they originate from the sub-hymenium as in *S. fuscum*. They are inflated below and are gradually narrowed towards the tip which never project beyond the basidial level.

Paraphyses.—These hymenial organs are often considered to be of great taxonomic value. These are generally unbranched but may often branch assuming peculiar form. The so-called 'Dendrophyses' as in *Asterostromella rhodospora* (Fig. 18) are narrow cylindrical hymenial organs which become much branched in a dichotomous manner. Sometimes they become narrow and cylindric and are thickly set with very short lateral branches all over, thus resembling a bottle-brush (*e.g.*, in *S. scytale*, Fig. 19 and *S. alternum*). Hence they are termed as 'bottle-brush paraphyses'. They may often branch at their tips (*e.g.*, in *Aleurodiscus* sp.) and are then known as the "antler type". They may also become flexuous as in *S. papyrinum*.

Hyphal pegs.—So far as the Bengal species are concerned these organs have only been met with in the case of *S. percome* (Fig. 20) in which they occur not uncommonly, yet never described by workers and seem to be of some taxonomic value. These organs are cylindrical in form, consisting of a cluster of closely agglutinated hyphæ which project beyond the level of the basidia. Burt records these organs in several *Thelephoraceæ* but more commonly in the *Polyporaceæ*.

S. umbrinum showing cystidia. Fig. 9. Cystidia of *S. papyrium*. Fig. 10. Vertical section of the fruit body of of *H. rubiginosa* showing setæ arising at different levels in the sub-hymenium. Fig. 11. Vertical section of the fruit-body of *H. aspera* showing setæ. Fig. 12. Hymenial layer of *H. tenuissima* showing straight, sharp pointed and conical setæ. Fig. 13. Hymenial layer of *H. nigricans* showing setæ with curved apices. Fig. 14. Vertical section of the fruit-body of *H. cacao* showing imbedded setæ.
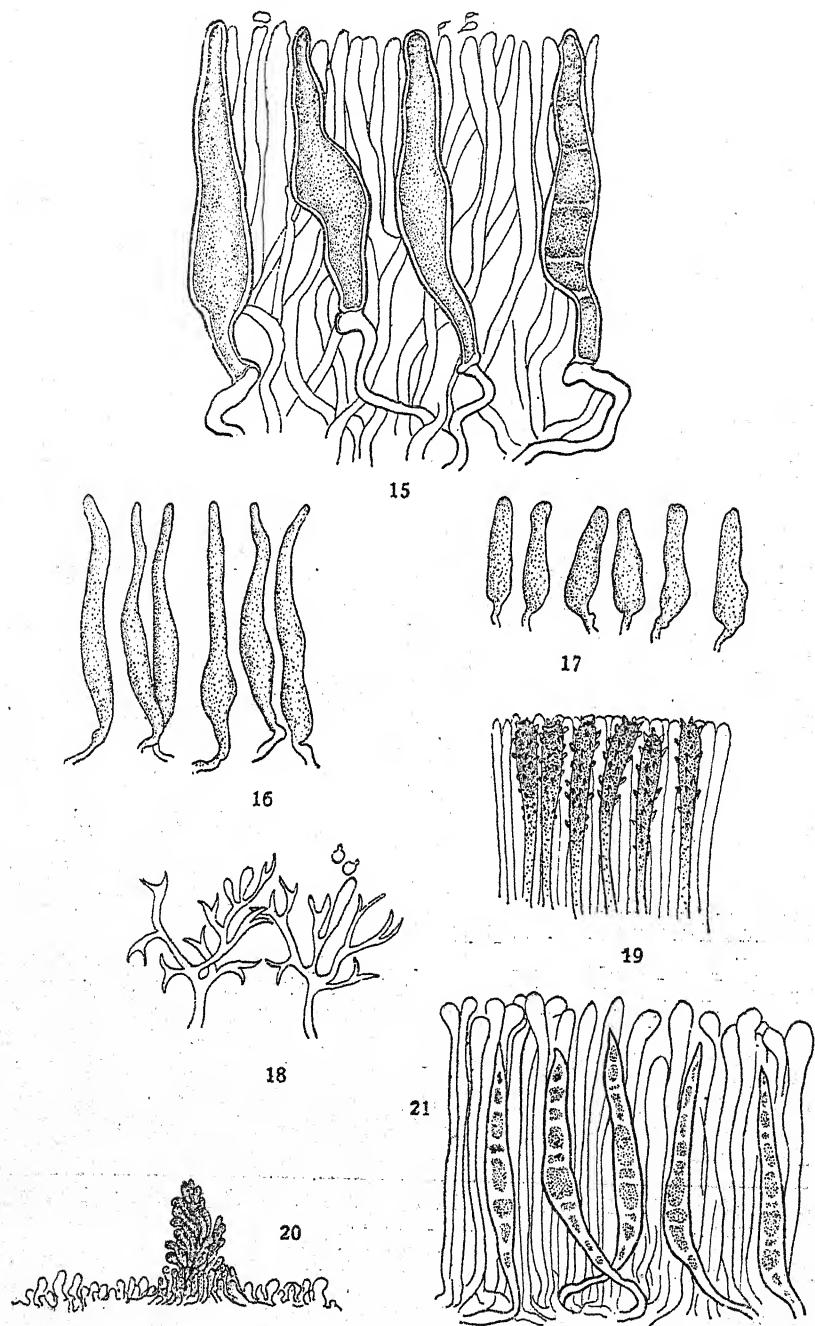
Figs. 15–21.—Fig. 15. Vertical section of the hymenial region of *S. fuscum* showing gleocystidia which are enlarged below and narrowed towards the apices; the shading indicates the dense opaque content

Lactiferous cells or *Conducting cells.*—So far as the Bengal species of *Thelephoraceœ* are concerned these organs have only been met with in the case of *S. hirsutum* (Fig. 21) where they occur as narrow elongated bodies curving upwards into the basidial layer.

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which in one case has been separated into sections due to its being dehydrated in glycerine. Figs. 16–17. Different types of gleocystidia in *S. nitidulum* and *S. petalades* respectively. Fig. 18. 'Dendrophyses' of *A. rhodospora.* Fig. 19. 'Bottle-brush' paraphyses of *S. scytale.* Fig. 20. Hyphal peg of *S. percome.* Fig. 21. Vertical section through the fruit-body of *S. hirstum* showing conducting cells with the broken up contents.

STUDIES IN THE PHYSIOLOGY OF RICE

II.   Photoperiodic response in one variety of winter paddy*
(A Preliminary Report)

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Introduction

Garner and Allard's (1920) investigations on the effect of the relative length of day and night on flowering, known as photoperiodism, have attracted the attention of numerous investigators. During the last two decades numerous investigations have been done in the West to find out the most favourable daylength for the flowering (photoperiod) of different plants and to elucidate the physiological processes involved in the photoperiodic response.   In accordance with their photoperiods, plants requiring light for less than 12 hours a day have been classified as shortday plants and as longday plants where the required light is more than 12 hours per day.   In the absence of a suitable photoperiod plants are found to continue vegetative growth and flowering is retarded or in some cases inhibited altogether.

The discovery of photoperiodism has proved to be of great help in determining the time for sowing of seeds of various crop plants in agricultural practice in the West.   Its utility lies in inducing late varieties to flower early by either prolonging or shortening the daylength artificially as the plant is a long- or shortday one.   Various workers in America, Europe and Russia have collected data for artificial control of flowering by this method.   Its practical application in agriculture is limited as the artificial increase or decrease in day length is not possible for plants growing in the open field.   One important fact that has emerged from this, however, is that for blooming a large number of plants have to pass through a stage of definite light period which is not connected with photosynthesis. As the prevalence of a definite light period depends on seasonal change of the year, the flowering of a plant is fixed according to the available day length.

Since this is not connected with photosynthesis, the problem presents itself whether the light stage (photostage) for the differentiation of floral parts could be supplied to the plant at an earlier stage

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* Paper read at a meeting of the Botanical Society of Bengal.

of its vegetative growth. This consideration has opened up new possibilities of accelerating plant development. The technique of accelerating plant development, as originally suggested by Lysenko for temperate cereals, is based on low temperature treatment of the seed before sowing and unless the plant passes this stage (thermo-stage) flowering is not possible. According to Lysenko low temperature during germination becomes the initiating factor in flower production. Recent investigations have shown that flowering is not always dependent on the action of low temperature. Purvis and Gregory (1937) have shown that flowering could be accelerated in winter rye without low temperature germination, but by a preliminary treatment of short days. They are of opinion that "If vernalization is defined as a treatment in early stages of growth which shortens the vegetative phase, one may speak legitimately of vernalization by shortdays as well as by low temperature." In recent years light vernalization has been practised in the West where low temperature treatment is without any effect. Cailahjan, Vasiljev and others have explored the possibilities of vernalization by light in U.S.S.R.

A brief review of vernalization by light as well as by low temperature has been recently published by this author (Sircar, 1939) wherein suggestions have been made for applying the technique to rice in Bengal. The sowing of winter paddy in Bengal is timely fixed as they flower only during a particular season of the year. Experiments conducted by Hedayetullah (1939) at the Government Rice Research Station at Chinsurah, have shown that flowering of rice variety *Bhasamanik*, is fixed in the third week of October (about the 20th October) irrespective of different dates of planting throughout the year. Seedlings which were transplanted in the 1st week of every month, excepting October and November, eared in every case in late October. From this it appears that the prevailing day length at the time of the year (shortday, *i.e.*, less than 12 hours sun light) has an influence on earing. In view of this an investigation was undertaken in this laboratory to induce earlier flowering by shortday treatment. The influence of the relative length of day and night on flowering of rice has not been properly studied in India. Hara (1930) and Fuke (1931) observed earlier flowering of some varieties of rice from Japan by photoperiodic treatment. Pan (1936) studied the length of exposure to light in relation to plant growth in rice. While crossing two varieties of rice which normally differ in flowering time, Alam and Saran (1938) noted the importance of relative daylength to induce earlier flowering. As has already been mentioned before (Sircar, 1939) that an investigation on the induced flowering of rice by shortday treatment will have an important bearing on problems of (i) vernalization of rice by light and (ii) crossing varieties of rice which normally flower at different daylengths. This paper presents the results of a preliminary work on the effect of shortday treatment on vegetative growth and flowering of winter paddy, variety *Bhasamanik*.

MATERIAL AND METHODS

A pure strain of rice, variety *Bhasamanik,* was used in this investigation. This particular variety was selected as it is a high yielding variety grown in Bengal and has been used before by the author for other physiological studies of rice (Sircar & Sen, 1941). The seeds were supplied by the Government Rice Research Station, at Chinsurah and were selected for uniformity of size and colour by eye. After sterilization with 0.2% formalin, 3 seeds were sown in each earthenware pot on June 29, 1940. The pots, $10'' \times 10''$, were filled with garden soil which was thoroughly mixed with one-eighth part by volume of cowdung manure. Germination of seeds was complete in course of a week. Usual agricultural practice of transplanting the seedlings from seed bed was not followed in this experiment. Of the three seedlings the best one was kept in each pot and the rest removed. In order to analyse the results statistically, a sufficient number of pots were used and the data for the individual pots were separately recorded. There were 50 pots in all, 25 pots for shortday treatment and 25 for the control (full day light). The plants were grown in the green-house of the Botany Department of Calcutta University.

Hara (1930) and Fuke (1931) working with some varieties of Japanese rice have recorded a suitable photoperiod of 8 hours for earlier heading. Fuke has further shown that in general, earlier heading is obtained by applying the treatment at an earlier stage of growth; for instance the treatment was much more efficacious when applied at the stage in which the plant bears 7 to 9 leaves and tillering becomes frequent. In this preliminary work only one shortday period of 8 hours from 8 A.M. to 4 P.M., was given to the plants when they had an average height of 65 cm. and 10 tillers per plant. Future experiments will be designed to make a comparative study of the photoperiodic effect of different daylengths on this and other strains of rice. For giving shortday, plants were exposed to the sun from 8 A.M. to 4 P.M. and for the rest of the day (*i. e.,* from 4 P.M. to 8 A.M.) they were removed to a well-ventilated light-proof house specially constructed for this purpose by the side of the green-house. The control plants were given normal daylight.

All other conditions except light treatment were kept, as far as practicable, identical in both sets of plants. The treatment was begun on August 8, 1940, and continued till the earing was noticed in the individual pots. In absence of any information on the photoperiodic response in rice in India, the duration of shortday exposure in this experiment was kept necessarily long so that an estimate of the effectiveness of the treatment could be made. In order to ascertain the minimal period of shortday exposure with an optimal effect of inducing earliness in rice, experiments after a statistical design will be carried out by the author in this season. Earliness in the number of days of ear emergence as an after-effect of shortday

treatment as compared to the control was studied and for this the
data for ear emergence of different plants were recorded.

EXPERIMENTAL RESULTS

Growth analysis.—In this experiment shortday treatment was
applied to plants when the seasonal day length was approximately
13 hours and rice plants in the field during this time show a rapid
vegetative growth. In order to test whether this reduction in day
length would affect growth and consequently the yield at harvest,
height measurement, tiller counts and the yield of the individual
plants were recorded. The significance of the treatment was statisti-
cally examined by the application of '*t*' test.

Height.—The height measurement adopted was the length of
the main shoot from its base to the tip of the highest leaf. The
mean values of height on three dates, given in Table I, show that as
compared with the control plants, reduction in daylight leads to a
small decrease in height which is not statistically significant. This
demonstrates that growth in height of the plant is not affected by
shortdays.

Tillering.—Tillers were counted on three dates ; the last count
was made when ear emergence was complete in all the tillers of the
individual plant. The figures obtained for tiller number include the
main shoot. The mean values recorded in Table I, show variations
due to treatment. With shortdays an increase in tiller from the
control is noticed. The statistical analysis of the data (Table I)
shows this is significant at the 5 p.c. level. The effect of shortdays
in having an increased number of fertile tillers as compared with the
control is noticed and statistically this is highly significant, $t = 3 \cdot 59$
being above 1% level of significance.

Tiller counts made at different dates show that all the tillers
of the control plants did not survive upto maturity, some of them
died, consequently there was a reduction in the number of tillers
bearing ears. The statistical analysis (Table II) shows that there
appears a just significant difference between tillers counted on
5–10–40 and 20–11–40 (ear-bearing tiller). On the other hand
with shortdays almost all the tillers were living upto maturity, a few
of these only appear to have been dead, but this difference will not
be statistically significant. From these results it is evident that
shortday treatment does not reduce the number of tillers per
plant, on the contrary it shows an increased number of fertile
tillers.

The date of ear emergence.—The criterion of ear emergence was
taken as the exsertion of the inflorescence through the last leaf
(flag leaf) sheath of the stem axis. For recording the dates of ear
emergence, observations were made each day at about the same
time, 3 P.M. The relevant data for ear emergence and the number

TABLE I

	Height in cm.			Tiller No.		
	19-9-40	30-9-40	24-10-40	25-9-40	5-10-40	Ear-bearing tillers
						8-11-40
Shortday (mean) ..	108·1	116·8	120·9	34·2	33·9	32·0
Standard error ..	±2·156	±1·323	±1·567	±1·574	±1·541	±1·369
						20-11-40
Control (mean) ..	113·07	116·78	123·82	29·32	29·38	25·6
Standard error ..	±1·205	±1·403	±1·591	±1·132	±1·102	±1·156
Difference of mean ..	−4·97	+0·02	−2·92	+4·88	+4·52	+6·4
Standard error of difference ..	±2·471	±1·929	±2·236	±1·939	±1·895	±1·781
Values of 't' ..	2·011	0·010	1·306	*2·516	*2·385	**3·593

* Indicates significance at 5 p.c. level.
** Indicates significance at 1 per cent. level.

TABLE II

Treatment	Date	Tiller No. (mean)	Standard error	Difference of mean	Standard error of difference	't'
Shortday	5–10–40	33·9	±1·541	1·9	±2·061	0·922
	8–11–40	32·0	±1·369			
Control	5–10–40	29·38	±1·102	3·78	±1·598	*2·365
	20–11–40	25·6	±1·156			

* Indicates significance at 5 p.c. level.

of days from sowing to earing are given in Table III. The data presented here denote the dates when earing was first visible in different pots. It will be evident that with the same date of sowing there is a marked difference in the dates of ear emergence in these two treatments (Figs. 1, 2, 3 and 4). Shortday exposure induces an average earliness in flowering by 20 days (Table III) ; the statistical analysis of the data shows that this effect is highly significant. An examination of the dates of earing of individual plants shows that a period of 13 days (Oct. 24 to Nov. 6) for the control plants and of 19 days (Sept. 25 to Oct. 14) for the shortday plants are required for the ear emergence of 25 plants in each treatment. This appears to be a fairly long period. Bell (1939) has noted that in a pure line normally developed plants will occupy a period of 4 to 5 days to accomplish ear emergence in a plot of 200–250 plants. Any variation in this period is attributed to weather conditions which may prolong or cause unevenness in earing in a pure strain. In field experiments this is unavoidable ; there will be always some plants which lag behind this period of earing by reason of some check to their growth and development. The present experiment with a pure strain, shows the earing period prolonged in both sets of plants, which appears to be due to some unknown climatic conditions. What is the precise nature of these is difficult to ascertain at this stage. Nevertheless the effect of shortday exposure in inducing earliness in this variety is very pronounced. That there results an earliness in earing of approximately 19–20 days by shortday period is evident from a consideration of the following fact. Bell and Ramiah (1933) have remarked that in a field experiment the satisfactory criterion of the date of ear emergence of a pure line population is the date at which approximately 50 % of the plants have come into ear. Table III shows about 50 % of shortday plants have come into ear on October 10, while in the control plants the corresponding date is october 29, thus showing an earliness of 19 days.

From the stage of ear emergence to ripening the period in the shortday plants is approximately 5 weeks and this is the usual time required in rice field for the ears to mature.

TABLE III

Shortday period			Control (full daylight)		
(8 A.M. to 4 P. M.)					No. of days from sowing to earing
Pot No.	Date of Ear emergence	No. of days from sowing to earing	Pot No.	Date of Ear emergence	
1	13–10–40	115	31	2–11–40	135
3	14–10–40	116	32	28–10–40	130
5	13–10–40	115	33	29–10–40	131
6	4–10–40	106	34	25–10–40	127
7	11–10–40	113	35	28–10–40	130
8	12–10–40	114	36	29–10–40	131
9	11–10–40	113	37	28–10–40	130
10	11–10–40	113	38	2–11–40	135
11	14–10–40	116	39	31–10–40	133
12	14–10–40	116	40	24–10–40	126
14	10–10–40	112	41	24–10–40	126
15	14–10–40	116	42	4–11–40	137
16	10–10–40	112	43	4–11–40	137
17	3–10–40	105	44	29–10–40	131
18	8–10–40	110	45	31–10–40	133
20	14–10–40	116	46	24–10–40	126
22	10–10–40	112	47	2–11–40	135
23	5–10–40	107	48	26–10–40	128
24	12–10–40	114	49	24–10–40	126
25	9–10–40	111	50	28–10–40	130
26	10–10–40	112	51	4–11–40	137
27	11–10–40	113	52	29–10–40	131
28	10–10–40	112	53	6–11–40	139
29	10–10–40	112	54	1–11–40	134
30	25—9–40	97	55	30–10–40	132
25 (pots)		111·9 (mean)	25 (pots)		131·6 (mean)

Standard error	± 08656	..	± 0·8029
Difference of means	..	..	19·7
Standard error of difference	..	..	±1·176
Value of ' t '	..	..	**16·75

Grain Yield.—The yield data are obtained from the weight of total grains of individual plants of shortday treatment. The ears after harvesting were dried in the sun for 3 days, and the grain weights were recorded. In these plants an average yield of 35·5 gm. per plant was noticed. Unfortunately the yield data for control plants are not available on account of the failure of seed formation in more than 50 % of plants. Ear emergence was complete in all the plants, later the grains did not set in a large number of plants. The exact cause of this is now difficult to trace. It is possibly due to some change in the cultural condition of control plants at the time of seed formation. In absence of the data for control plants, a comparison with the yield of rice plants of Chinsurah rice research station may be made.

The figure shows an average yield of 31 gm. per 25 plants, while the yield obtained in the shortday plants is 35·5 gm. an average of 25 plants. From this it is evident that the photoperiodic treatment as applied to these plants has not reduced the grain yield.

DISCUSSION AND CONCLUSION

The result presented in this paper indicates the importance of the relative length of day and night as a factor in initiating flowering in winter paddy, variety ' *Bhasamanik* '. An exposure of 8 hours sunlight to the plants at an earlier stage of vegetative growth has accelerated ear emergence by approximately 20 days. This demonstrates that for flowering, these plants are to pass through a photostage of short illumination, in other words the variety is a shortday plant according to Garner and Allard's classification. The treatment applied to the plants at the stage of rapid vegetative growth had no adverse effect on height and tillering. On the contrary an increase in tiller number was noticed ; almost all the tillers survived upto maturity and became fertile while death of some tillers was observed in the control plants, consequently the number of ear-bearing tillers is diminished. Height differences between the control and the treated do not appear significant. Grain yield is not reduced, on the other hand an indication of increased yield is obtained.

In this preliminary work the effect of only one shortday treatment for a long duration (till the earing was visible in respective pots) was studied, so a full discussion on the problem of photoperiodic response in rice cannot be undertaken with a limited number of observations. Looking into the extensive literature published on the photoperiodic studies in different plants it is realised that a great deal of data would be necessary to throw further light on photo periodic response.

Physiological processes involved in the photoperiodic effects are not well understood. The need of a suitable photoperiod for the initiation of flower meristem has been postulated by some investigators (Cailahjan, 1936, Hamner and Bonner, 1938) as the physiological pre-requisites for the synthesis of a specific flower-forming

hormone, called florigen. This substance is assumed to be manufactured in the leaves and moves to various parts resulting in floral initiation and subsequent development. It has been claimed by Cailahjan (1936), that plants respond photoperiodically as soon as the first green leaf is unfolded. Cailahjan has further shown that shortday treatments applied to millet were most effective when the plants were one to five weeks' old and that it was at these periods millet made its rapid growth. Borthwick and Parker (1938) have shown the effectiveness of photoperiodic treatment just after the green leaves are formed in Biloxi Soybeans.

The hypothesis advanced by Cailahjan and others may have an important bearing on rice culture. Perception of photoperiodic stimuli to synthesize florigen or some such substance in the seedling condition to induce earlier flowering will have practical application in the cultivation of transplanting rice. The present investigation has shown induced flowering of rice variety ' *Bhasamanik* ' by shortday exposure. The problem now awaits to investigate the possible use of the results in agricultural practice.

SUMMARY

A pot culture experiment on photoperiodic response in winter paddy variety *Bhasamanik*, is described. There were two treatments (i) shortday and (ii) full-day (control).

Shortday treatment of 8 hours Sunlight was applied to the plants at the stage of rapid vegetative growth and continued till earing was noticed. Reduction in day length had no adverse effect on vegetative growth and grain yield; on the other hand a significant increase in tiller number was noticed.

As compared with the control plants, an earliness in ear emergence of approximately 20 days (average of 25 plants) was noticed in plants receiving shortdays. The results were statistically significant.

The bearing of these results on agricultural practice is discussed.

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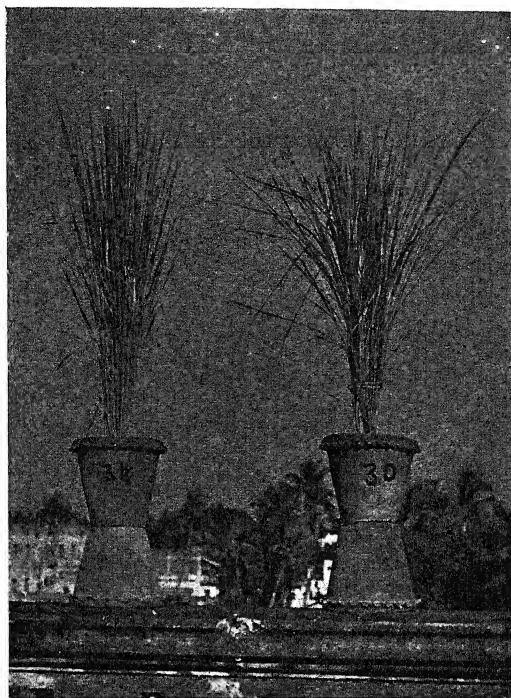
Fig. 1.   Shows the ear emergence of one plant (pot 30) from
            short day treatment, while the control one is without
            ears out.

Photographed on 2-10-40.

Fig. 2.   Ear emergence of two plants (pots 17 and 6) is shown,
            control ones have not yet eared.

Photographed on 4-10-40.

S. M. SIRCAR—*STUDIES IN THE PHYSIOLOGY OF RICE, II*
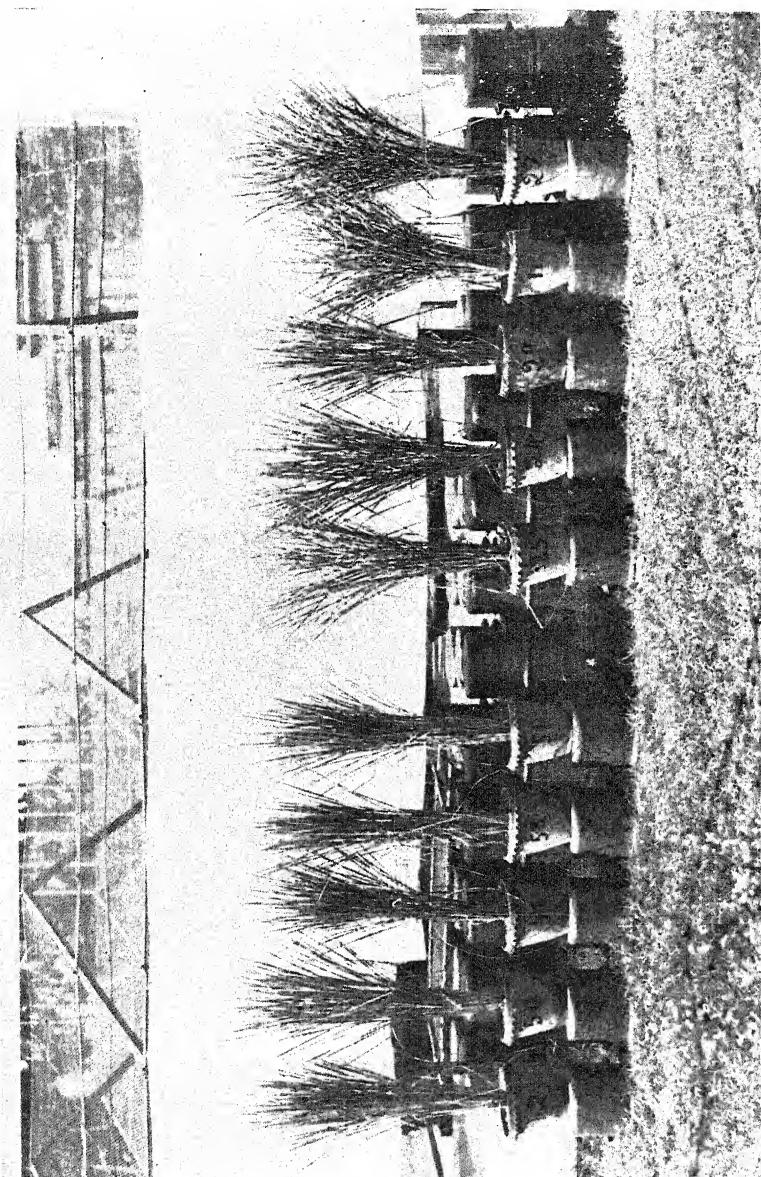
Fig. 3. Plants (pots 11, 20, 22, 24 and 25) from shortday exposure show ear emergence. Control ones are without ears.

Photographed on 24-10-40.
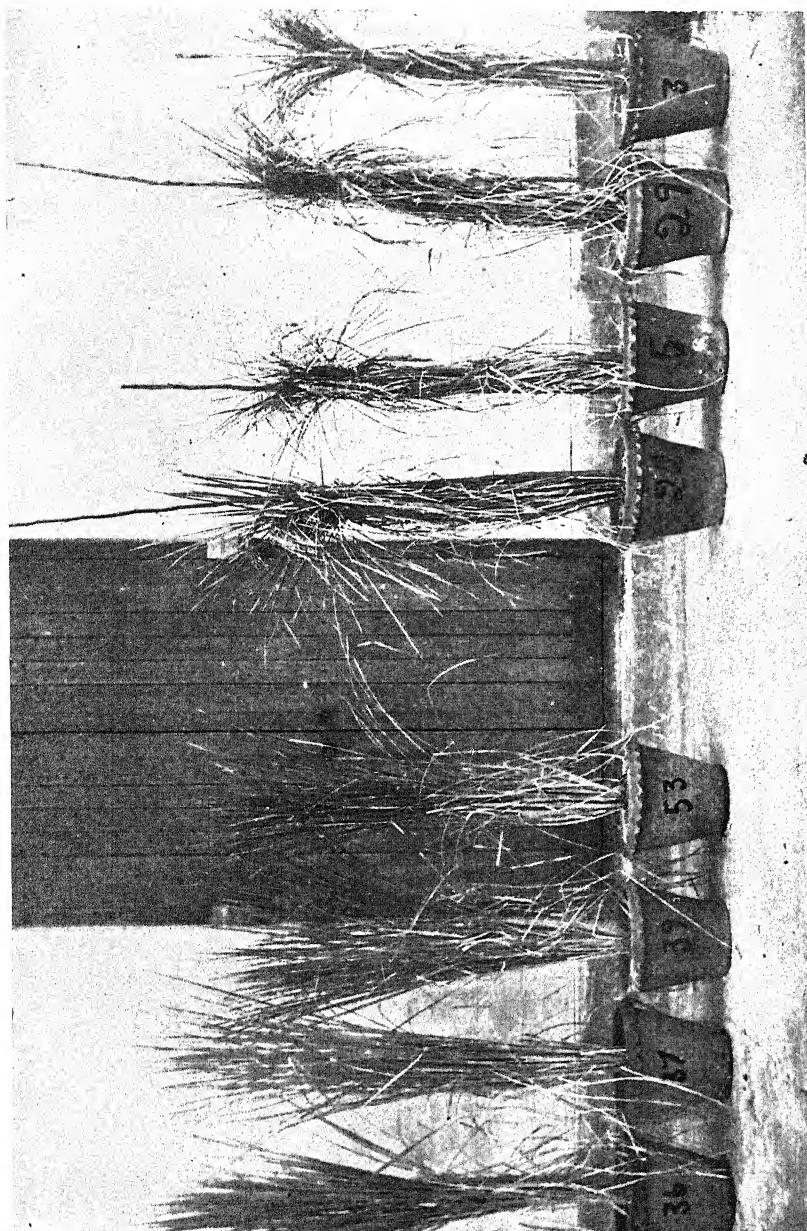
Fig. 4. Plants from shortday treatment are ready for harvest, while ears have just emerged from Control plants. An earliness of 20 days is observed in the treated plants. Photographed on 14-11-40.

S. M. SIRCAR—*STUDIES IN THE PHYSIOLOGY OF RICE, II*
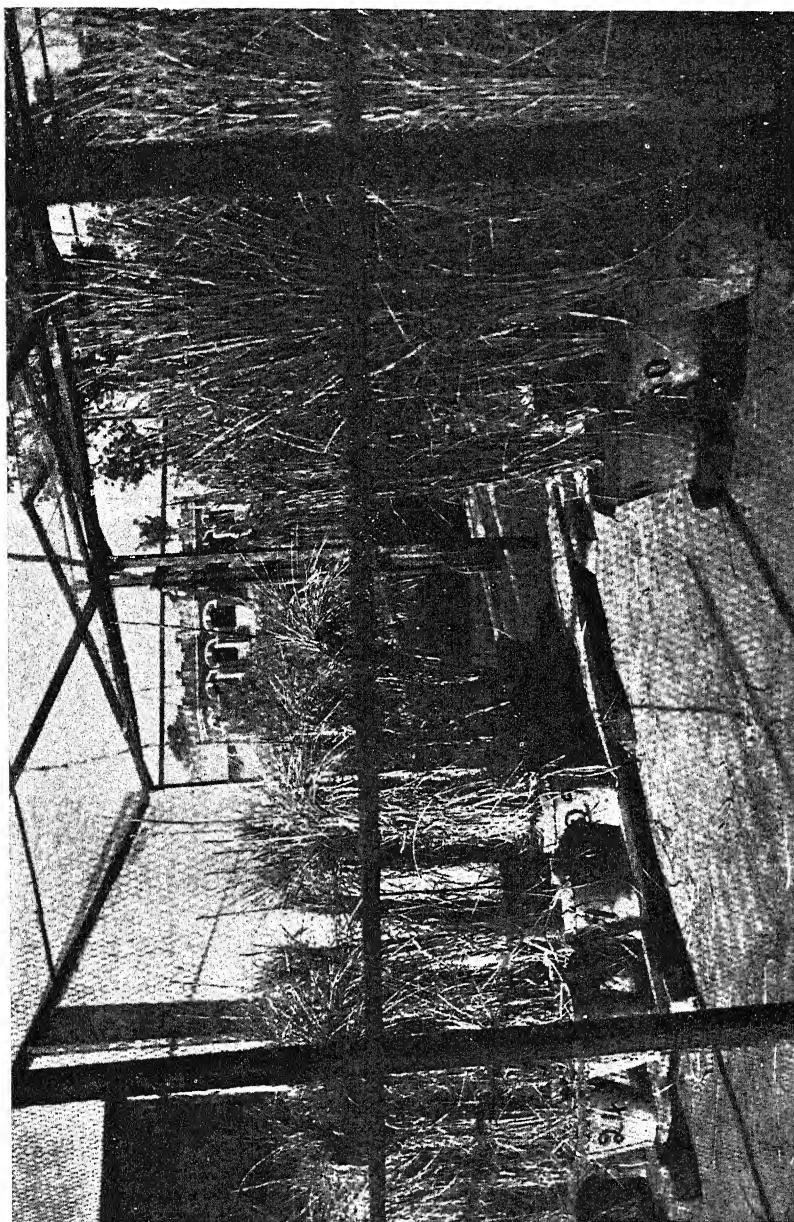
Fig. 5. Shows the green house arrangement and the short day treated plants are ready for harvest. Control ones have ears out only.

Photographed on 15-11-40.

DEVELOPMENT OF EMBRYO-SAC AND ENDOSPERM-HAUSTORIA IN *REHMANNIA ANGULATA*, HEMSL.

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SOME papers on the formation of Embryo-sac and Endosperm haustoria in several members of Scrophularineæ have been already published by the author (Krishna Iyengar, 1937, 1939*a, b* and *c*, 1940*a, b* and *c*, 1941*b*). A paper on *Tetranema mexicanum* was read during the Indian Science Congress session of 1941 (Krishna Iyengar, 1941*a*), and the same was combined with *Verbascum* as a paper in the series (1941*b*) on account of several points of affinity. Although there are a few abnormalities common to *Tetranema* and *Rehmannia* the latter had to be treated separately on account of several points of variation as explained in the following account.

MATERIAL AND METHODS

The material for investigation was collected from the Government Gardens at Ootacamund, and fixed in Nawaschin's fixative. The sections were cut between 8 and 12 μ, according to the stages required, and stained in Heidenhain's iron-alum hæmatoxylin.

OVULE

The ovule consists of a single integument and a reduced nucellus having a large hypodermal archesporial cell (which directly becomes the megaspore mother cell) surrounded by a layer of jacket cells. During the development of the ovule very little starch is present in the cells. The innermost layer of the integument forms the tapetum with rich protoplasmic contents. The hypostase is present but it does not attain the significant development so characteristic of *Celsia, Isoplexis* (Krishna Iyengar, 1939*a*) and *Verbascum* (Krishna Iyengar, 1941*b*). There are some large cells with prominent nuclei scattered in the placenta and the integument but they do not undergo any appreciable change in post-fertilisation stages and hence appear to be without any nutritive function.

DEVELOPMENT OF THE EMBRYO-SAC

The megaspore mother cell gives rise to a linear tetrad of megaspores. During its formation it was noticed that in several cases the upper dyad shows only nuclear division without wall formation (Fig. 1). Thus there are only three cells, the uppermost being bi-nucleate. The chalazal megaspore gives rise to a normal eight-nucleate embryo-sac as in Fig. 2 while the others degenerate. The

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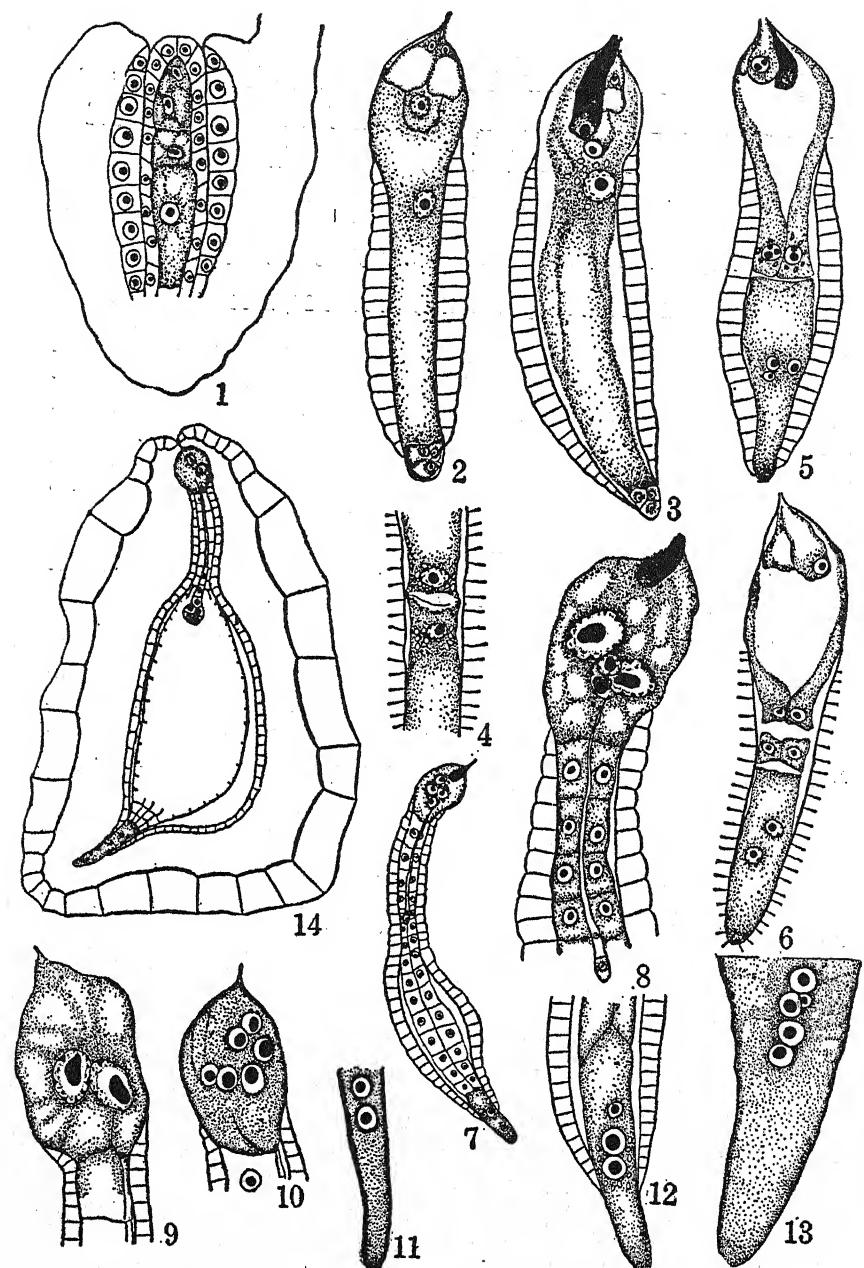
Figs. 1–14. Fig. 1. Linear tetrad formation (× 480). Fig. 2. Embryo-sac (× 365). Fig. 3. Embryo-sac after fertilisation (× 320). Fig. 4. First division of the primary endosperm nucleus (× 320). Fig. 5. Second division of the primary endosperm nucleus (× 320). Fig. 6. Third division of the

sac is enlarged towards the micropyle and narrow towards the chalaza. The egg apparatus is composed of two large synergids and the egg, the former having a small nucleus and a large vacuole. The antipodals are small and the polar nuclei fuse to form a secondary nucleus which moves upward and lies just below the egg. There is a considerable deposition of starch in the micropylar portion of the integument, as well as in the mature embryo-sac. The nucellar jacket begins to degenerate as the embryo-sac matures and only its insignificant remains are present when the sac is fully formed.

Stages in fertilisation have not been studied. One of the synergids seems to be destroyed by the pollen tube during its passage into the embryo-sac (Fig. 3). The antipodals begin to degenerate from this time onwards.

DEVELOPMENT OF ENDOSPERM AND EMBRYO

The first division of the primary endosperm nucleus is transverse, and this is followed by a wall resulting in the chalazal and micropylar chambers (Fig. 4). The next division which is longitudinal is confined to the micropylar chamber, while only a nuclear division takes place in the chalazal chamber (Fig. 5). The third division is transverse and this separates two middle cells from the micropylar tier (Fig. 6); the former undergo a series of transverse divisions giving rise to the endosperm, while the two micropylar cells and the chalazal chamber develop into the haustoria. The fully formed endosperm is differentiated into a specialised tissue towards the two ends for the taking in of food materials from the haustoria to the more deeply placed storage tissue (Fig. 14). Towards the micropylar side some of the conducting cells enlarge significantly and may themselves serve in the absorption of nutrition from the surrounding integumentary cells and thus assist the micropylar haustorium. But the major rôle is certainly the transportation of nutrition from the haustorium to the endosperm proper. The endosperm tissue while it is being formed does not have any starch in the beginning, although this is quite prominent in older stages as well as in the mature embryo-sac. Thus it is probable that the starch in the embryo-sac is utilised during the early stages in the formation of endosperm tissue and might as well indicate a probable delay in the haustorial action.

Some of the stages in the development of the embryo have been studied, and these indicate that the embryo is of the *Capsella*-type.

ENDOSPERM HAUSTORIA

The chalazal chamber directly develops into a haustorium. At times an incomplete longitudinal wall is present in this chamber

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primary endosperm nucleus ($\times$ 320). Fig. 7. Long. section showing endosperm haustoria ($\times$ 97·5). Figs. 8–10. Micropylar haustoria showing variation in nuclear number ($\times$ 320). Figs. 11–13. Several stages of growth of chalazal haustorium (Figs. 11 and 12. $\times$ 320; Fig. 13. $\times$ 730). Fig. 14. Longitudinal section of a young seed ($\times$ 65).

(Fig. 13) indicating its derivation from an originally two-celled structure. The nuclear number varies   (Figs. 5, 6, 11–13). Often the haustorium will be bi-nucleate, one of the nuclei being smaller and placed at times towards the chalaza, but occasionally the number ranges from three to five, one of the nuclei being much smaller than others. This haustorium is simple and non-aggressive and without any elaborate differentiation.

The micropylar haustoria develop from the two cells cut off towards the micropyle.   The separating membrane is very thin and its early dissolution results in the formation of a single bulbous body with 2–6 nuclei (Figs. 8–10).   Although non-aggressive, it yet shows a high degree of haustorial action as evidenced by its large nuclei and rich contents and its accumulation of nutritive material in its neighbourhood.   Older haustoria show hypertrophied and amœboid nuclei of various sizes.

INTEGUMENTARY TAPETUM

This layer is differentiated from the integument at a very early stage and is composed of large cells with rich protoplasmic contents and prominent nuclei.   During further growth of the embryo-sac it becomes confined only to its non-dilated lower part of the sac. While the endosperm is being formed this layer loses most of its contents, becomes reduced in size and develops a thick cuticle. Some of the cells towards the micropyle are however not so much affected, and do not show any significant cuticle formation, but maintain their thin-walled nature.   This feature makes one suspect that to a certain extent the nutritive material of the integument enters inwards through this region also.

DISCUSSION

Rehmannia conforms to the other members in the development of the embryo-sac from the innermost megaspore of the linear tetrad, but instead of four megaspores being formed only three are usually present, the outermost being bi-nucleate. A similar stage, though with the bi-nucleate megaspore towards the chalaza, has been reported by Schmid (1906) in *Pedicularis verticillata*. A deposition of starch in the integument as well as in the embryo-sac is noticed not only in this plant but also in *Celsia, Isoplexis* (Krishna Iyengar, 1939a), *Tetranema, Verbascum* (Krishna Iyengar, 1941b) and others. In *Celsia* the nucellar jacket towards the micropyle also stores up starch during the development of the embryo-sac. A hypostase is present in *Rehmannia* although the radiating arrangement of cells so characteristic of *Celsia* and others, is wanting.

Endosperm and haustoria.—Just as in the other members of the family the early divisions of the primary endosperm nucleus result in the organisation of the two kinds of haustoria towards the ends and endosperm tissue in the middle. There is the usual differentiation of the latter into the conducting and the storage tissue. Some of the conducting cells towards micropyle enlarge just as in *Alonsoa*

(Krishna Iyengar, 1937), *Tetranema* (Krishna Iyengar, 1941*b*) and others, and this feature combined with the thin-walled nature of the tapetum at this end indicate that they probably have a partially haustorial function (either direct or indirect) besides the transportation of the nutritive material from the haustoria to the endosperm tissue. In *Verbascum thapsus* (Krishna Iyengar, 1941*b*) the conducting cells at the chalazal end enlarge and are probably similar in function.

The chalazal haustorium is the earlier to be organised as also to degenerate.   Its non-aggressive nature is comparable to *Tetranema* while its incomplete partitional wall reminds one of *Paulownia* (Millsaps, 1936) and *Veronica* VI type (Glišić, 1936–37).   The varying number of nuclei and their different sizes also find a parallel in *Tetranema*, although in other respects these two genera are much related to each other.   This multinucleate chalazal chamber can be compared to the ' basal apparatus ' (Schnarf, 1929) met with in some other families.

At the micropylar end a bi-nucleate haustorial body is formed by the dissolution of the separating membrane between the two cells originally laid down.   Occasionally a tri- or a tetra-nucleate haustorium is met with, while in one instance as many as six nuclei were seen.   The presence of 4 or more nuclei in the haustorium is quite a common feature in the Labiatæ (Junell, 1937; and Narasimha Murthy, 1940).

The tendency to reduce the number of haustorial cells and their nuclei happens to be a general feature of the Scrophularineæ.   The response to the nutritional stimulus often results in the hypertrophy of a cell or its nucleus or both, but the enlargement of one of the two nuclei of the chalazal haustorium, and the degeneration of the other nucleus which is nearer to the chalaza and thus directly placed in the way of the nutritional stream (as seen in *Vandellia*, Krishna Iyengar, 1940 *a*) make one doubt if all the nuclei of a haustorium are endowed with the same responsive capacity.

Hypertrophy of a nucleus is often interpreted by some as a pathological condition.   As opposed to this view there are many instances where hypertrophied nuclei persist till a late stage is reached, and only then begin to show greater accumulation of chromatin material, loss of proper differentiation, dissolution of the nuclear membrane and a final break up.   Since the greatest development of the haustorium in most of these members coincides with the hypertrophy of its nucleus, it may reasonably be concluded that this condition is the healthy result of a response to the nutritional stimulus and expressive of a high activity of the nucleus instead of being an indication of a state of malady or senility.

SUMMARY

1. *In Rehmannia angulata* the large hypodermal cell directly functions as the megaspore mother cell, from which a linear tetrad

of megaspore is often formed. At times there are only three spores, the outermost one (towards the micropyle) being bi-nucleate.

2. A normal eight-nucleate embryo-sac develops from the chalazal megaspore. The synergids are large and the two polar nuclei fuse to form a secondary nucleus which moves up just below the egg.

3. The integumentary tapetum sheathes only the non-dilated chalazal part of the sac. It loses most of its cell-contents during the development of the endosperm and embryo.

4. The first three divisions of the primary endosperm nucleus result in the micropylar and the middle tiers of cells with two cells in each tier, and the chalazal chamber with or without an incomplete partitional wall. The cells of the middle tier give rise to the endosperm while the haustoria are organised at the two ends.

5. Some of the conducting cells of the endosperm towards the micropyle are enlarged and probably assist in the absorption of the tissue contents of the integument.

6. The non-aggressive chalazal haustorium is often bi-nucleate but occasionally 3, 4 and 5 nuclei are also met with, one of the nuclei often being much smaller than the rest.

7. The micropylar haustorium is composed of two uni-nucleate cells, but the early dissolution of the separating membrane results in a single bi-nucleate body which is bulbous and non-aggressive. Even here the nuclear number occasionally varies from 4 to 6. The nuclei are amœboid and hypertrophied when the haustorium is mature.

8. The development of the embryo is of the *Capsella*-type.

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MORPHOLOGY AND PARASITISM OF *TROCHODIUM SAMPATHENSE* THIRUMALACHAR, SP. NOV.

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THE genus *Trochodium* was founded by Sydow (1919) to accommodate a rust parasitic on the leaves of *Ipomoea argyreoides*, Chois. in South Africa. Thümen (1878) who first observed it thought that two rusts were involved and he designated the aecial stage *Aecidium Ipomoeae* and the uredal stage *Uredo aterrima*. Berkeley (1882) who also examined it concluded however that there were not two rusts but two stages of one and the same rust, and renamed it *Uromyces Ipomoeae*. Sydow (1919) thought however that the peculiar configuration of the teliospores justified the erection of a new genus to which he gave the name *Trochodium*, and the genus has been accepted by Dietel (1928), Saccardo (1925) and Clements and Shear (1931) though Doidge (1926) retains the name *Uromyces Ipomoeae* in her monograph of the South African rusts. The rust has so far been recorded only from South Africa and the genus is monotypic.

Collections of rusts on *Argyreia speciosa*, Sw., *A. cymosa*, Sw., and *A. argentea* and on *Lettsomia elliptica*, Wight. made in Assam, Madras Presidency and the Mysore State respectively have been identified by Sydow and Butler (1906, 1907) to be *Aecidium Argyreiae* Berk and Broome, but collections of rusted specimens of *Argyreia cymosa* at Hiriyur and *Lettsomia elliptica* at Bangalore made by the author in December 1940 have shown the telial stages possibly of this same rust. Culture experiments which were subsequently carried out have shown this surmise to be correct. The rust is a new species belonging to the genus *Trochodium* to which the author proposes to give the name *Trochodium Sampathense*.

SYMPTOMS

The fungus attacks leaves, twigs and fruits of the host plant, causing slight swellings (Figs. 16, 17). The infection, beginning as a tiny yellow speck, spreads gradually forming concentric yellow patches.

It assumes an active phase soon after the rains in August, and pycnia which are evanescent are developed in large numbers along with aecia. Due to secondary infection by the aeciospores, the infection very soon spreads to the adjacent plants. During the

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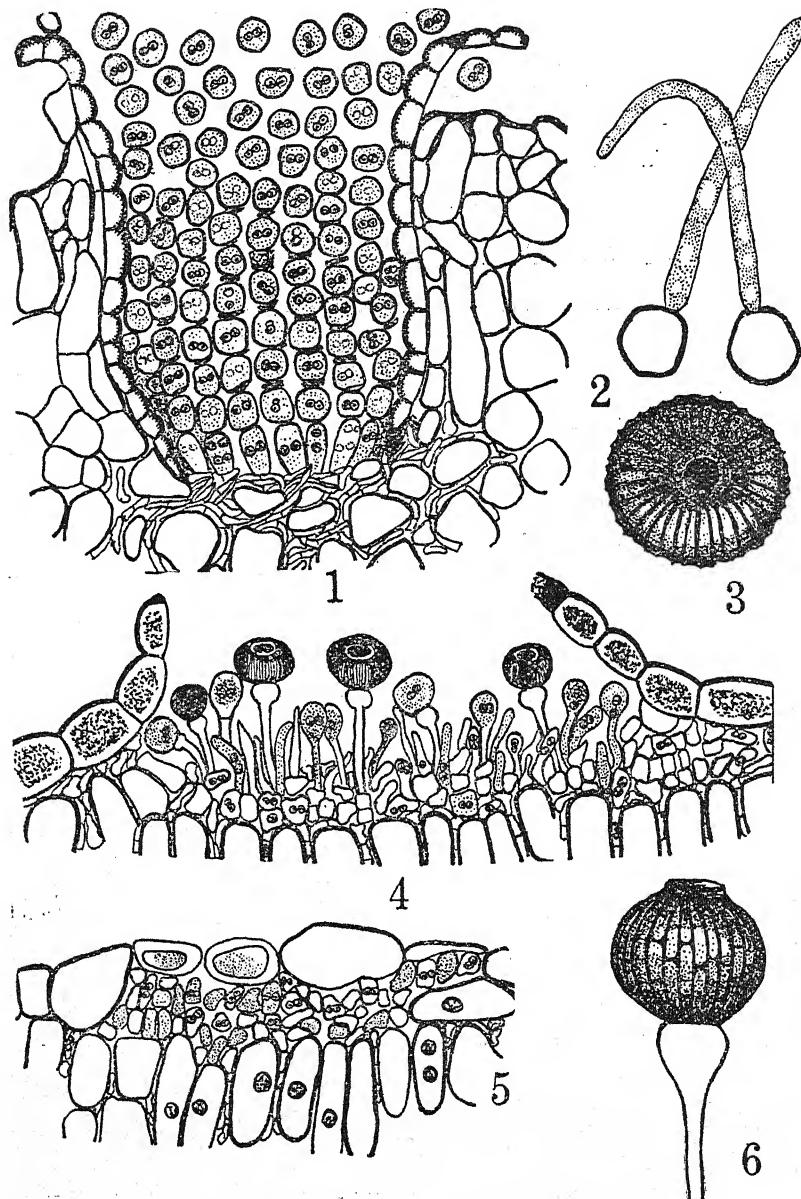
Figs. 1–6.—Fig. 1. Camera lucida drawing of an aecium on *Argyreia cymosa*, showing chains of spores and peridia (× 300). Fig. 2. Germination of aeciospores (× 400). Fig. 3. Surface view of the teliospore showing the central pit with the striations of the exospore converging around it (× 900). Fig. 4. Transverse section of the teliosorus showing spores in various stages of development (× 400). Fig. 5. Showing the initials of telia formed beneath the epidermis (× 400). Fig. 6. Teleutospore showing ribbed exospore and inflated stalk (× 900).

months of September, October and November, only secondary
aecia are produced, but after the second week of December, telia
are formed as black specks on the infected patches. Telial sori
present a fluffy powdery appearance when examined with a field
lens.

Material for microscopic studies was fixed in Allen's modifica-
tion of Bouin's fluid, and Formalin acetic alcohol. Sections 8 to 10μ
thick were cut and stained with Haidenhain's iron-alum hæmatoxy-
lin, with Orange G as counterstain. Material for spore germination
was stored in paper bags in a cool place. Spores were germinated
and stained by the method previously described by the author
(1940).

TELIA

· Telia which develop in the month of December are the winter
spores. They are amphigenous and chestnut brown to black in
colour. Initials of telia are developed beneath the epidermis, the
mycelium being binucleate (Fig. 5). From them emerge slender
hyphæ, at the tips of which the spores are abstricted. The nuclei
migrate into the spore and are cut off by a wall. The stipe of the
spore enlarges in size due to inflation (Fig. 4).

Sydow (1919) described the teliospores of *Trochodium Ipomoeae*
(Thum) Syd. as being unicellular, stipitate and deep brown in
colour, with an inflated stalk. The wall of the spore is ribbed around a
central pit. Teliospores of the present *Trochodium* are also deep
brown in colour, amphigenous and erumpent. In early stages the
spores are pale yellow and binucleate (Fig. 12), but mature spores
are uninucleate due to the fusion of the nuclei (Fig. 13). The spores
are spherical with a rounded apex. The exospore is thick with longi-
tudinal striæ, which radiate from the apex and converge towards
the base. The apical germ pore is situated in a pit, and is conspi-
cuously thickened along the margin (Fig. 3). The spores are not
shed at maturity but remain attached to the sorus.

It is interesting to note that in *Trochodium Ipomocae* described
by Sydow and figured by Dietel (1928) the spores have stalks which
are inflated to a size bigger than the spore itself. This however
is not the case in the *Trochodium* under study, for, the stalk in this
case is inflated only at the place of attchment, and is smaller in size
than the spore. The spores are not applanate as in *Trochodium
Ipomoeae* Syd. but almost spherical. They measure $20 \times 26 \mu$.

Fresh spores collected during the months of March and April
did not germinate, indicating that a period of rest was necessary.
Teliospore material stored in the laboratory was tested from time to
time for germination. Teliospores after a rest period of three to four
months germinated however quite readily.

The process of germination is as follows :—a single nucleus
migrates from the teliospore into the germ tube (Fig. 11). The four
cells of the promycelium which is formed are uninucleate (Fig. 8).
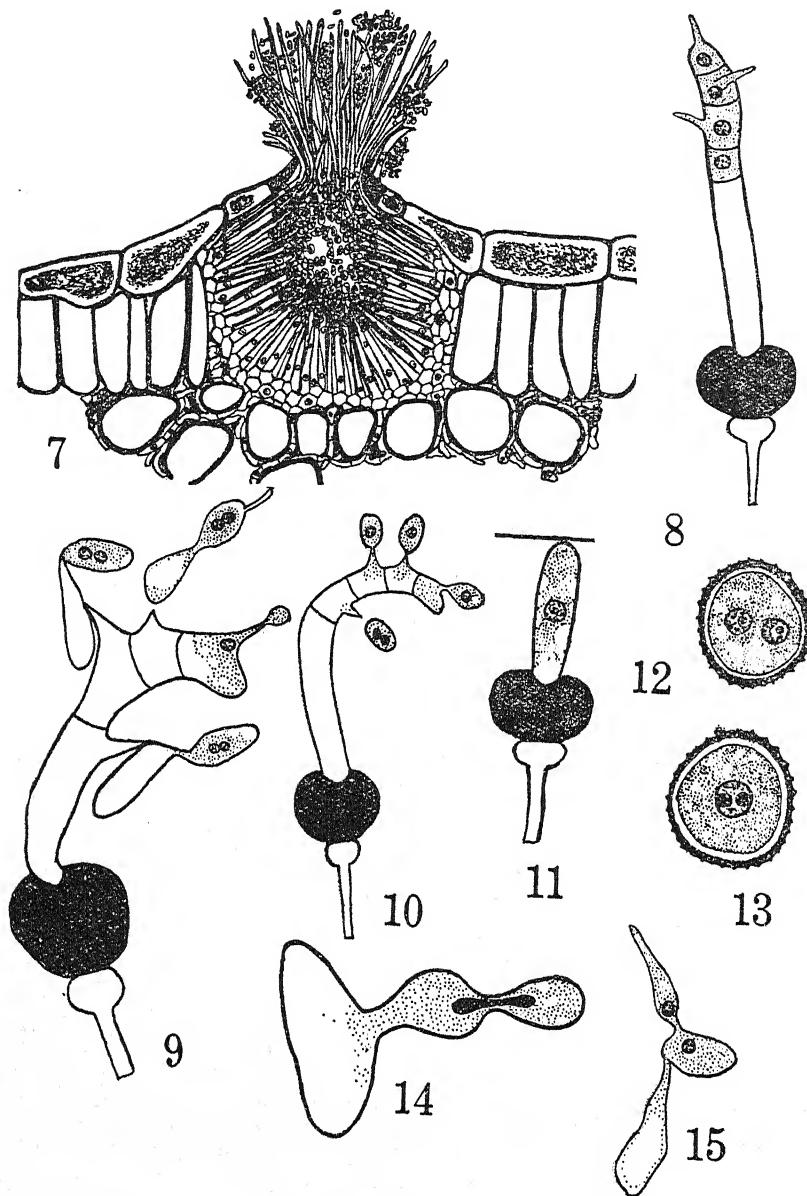
Figs. 7–15.—Fig. 7. Camera lucida drawing of a pycnium with ostiole and paraphyses (× 400). Fig. 8. Four-celled promycelium showing the development of sterigmata (× 400). Fig. 9. Germinating teliospore showing the formation of secondary sporidia (× 800). Fig. 10. Showing the development of sporidia (× 400). Fig. 11. Germinating teliospore with one-celled promycelium (× 400). Fig. 12. Cross section of a young teliospore showing two nuclei. (× 800). Fig. 13. Early stage of

Basidiospores are formed at the tips of the sterigmata (Fig. 10). These are spherical, thin-walled and uninucleate in early stages. In most of the cases observed the mature spores after abscission showed binucleate condition. The formation of secondary sporidia is of common occurrence. The nucleus and the contents of the primary sporidium migrated into the secondary sporidium even while attached to the sterigma (Figs. 9 and 19). The development of secondary sporidia was not affected by moisture conditions, and seemed to be a normal phenomenon. The secondary sporidium is always smaller than the primary sporidium which retains its shape as a balloon-shaped structure.

Secondary sporidia are commonly met with among rusts, and various investigators have recorded their occurrence. They have been found to develop in *Cronartium ribicola* Fischer by Colley (1918), *Phragmidium* sp., *Uromyces Hobsoni* Vize., and other rusts. Blackman (1904) states that no significance can be attached to the development of secondary sporidia. However in this *Trochodium* the development of secondary sporidia is normal and is not affected by moisture conditions. Perhaps the empty primary sporidia are in the nature of balloon-shaped bodies functioning as parachutes for fecilitating the dispersal of sporidia to long distances. Development of tertiary sporidia (Fig. 15) was also observed in a few cases. The nucleus becomes elongated in the process of migration (Fig. 14). Similar instances of tertiary sporidia formation have been reported in *Uromyces Hobsoni* by the writer (1939).

Aecia

Aecia are not restricted to the lower surface of the leaf, but are found to be distributed on either surfaces. They are cupulate and covered with well-developed peridia (Fig. 1). The aecial plectenchyma is sub-hypodermal, formed by the concentration of hyphæ in the intercellular spaces. Aeciospore mother cells are abstricted from the basal cells. The outermost layers of the aecial chain become transformed into the peridial layers. The aeciospores are polyhydral, yellow in colour, minutely spinescent, with an indistinct germ-pore. The spores measure $20 \times 17 \mu$. The spores readily germinate within 24 hours, and develop very long germ tubes if allowed to remain in the germination chamber for a long time (Fig. 2).

Pycnia

Pycnia which have not so far been recorded for the genus *Trochodium* are being now reported for the first time. In the course

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nuclear fusion in the teliospore ($\times$ 800). Fig. 14. " Fig. 16–19 " and Camera lucida drawing showing the migration of the nucleus from secondary sporidium to tertiary sporidium ($\times$ 1800). Fig. 15. Showing the development of the tertiary sporidium ($\times$ 800). Fig. 16. Photograph of an infected leaf of *Argyreia cymosa* (about natural size). Fig. 17. Infected leaf of *Lettsomia elliptica* ($\times \frac{1}{2}$ natural size). Fig. 18. Photomicrograph of the pycnium ($\times$ 280). Fig. 19. Photomicrograph of a germinating teliospore showing the formation of secondary sporidia ($\times$ 500).

of the studies the author observed a few pycnia in section and later they were made out in large numbers even macroscopically. They are amphigenous, and appear as orange coloured specks when examined with a lens. They are flask-shaped sub-hypodermal, with an ostiole. Paraphyses and flexuous hyphæ are exserted from the ostiole enmeshed in nectar containing spores (Figs. 7, 18). The initials of pycnia are also sub-hypodermal, the mycelia being concentrated in the intercellular spaces. The hyphæ are uninucleate. Pycnospores are oval or spherical in shape with a large nucleus, which is vacuolate.

AECIOSPORE INFECTION

Host plants of *Argyreia cymosa* and *Lettsomia elliptica* were raised from seeds under rust-free conditions. The plants were kept in glass cages with adequate aeration. Fresh aeciospores collected from the respective hosts, were used for infecting the plants, since spores stored in paper bags lost their viability after two weeks. Young leaves to be infected were sprayed with water and dusted with the aeciospores. Small portions of infected leaves of the host plant with freshly erupted aecia were gently rubbed on the undersurface of the leaves of the host plant, which had been previously sprayed with water. This method of infection was found to be quite useful by Anderson (1939) for infecting currant leaves with urediospores of *Cronartium ribicola*. The infected plants were enclosed for 48 hours in moist chambers, inside which a relatively high humidity was maintained.

The first sign of infection became perceptible after 8 to 7 days, as a small yellow speck, and this gradually spread out. The infected portions showed slight hypertrophy, and after 15 or 20 days, aecial pustules were formed on these yellow patches. The epidermis was ruptured by the developing chains of spores. The infection caused by the secondary aecia was found to be very virulent. The infected patches rapidly dry up, and hasten the defoliation of the plant. The secondary infection of aeciospores in *Trochodium* is associated with the absence of uredia in the life-cycle of the rust. Barclay (1895) in discussing the secondary infections of aeciospores in *Uromyces Hobsoni* where a similar phenomenon occurs, states, " the assumption by the aecidiospores of the function of urediospores, and the consequent non-necessity for the production of the latter is significant ".

SPORIDIAL INFECTION

Teleutospores were germinated on slides in moist chambers. *Argyreia cymosa* plants grown under rust-free conditions were inoculated with sporidia. For the purpose of localizing the infection, small squares were outlined on the leaf with water-proof Indian ink. The inoculum was placed after spraying the leaves with sterile tap water. The infected plants were kept in moist chambers for 24 hours. Eight days after inoculation, small yellow spots were

observed on the leaves indicating successful infection. The spot gradually enlarged into a patch. Pycnia were observed after 15 days developing as orange coloured specks, with tiny drops of nectar. The pycnia are evanescent, and degenerate after 7 to 9 days. The development of aecia follows that of pycnia. The infected patch becomes studded with aecial pustules. The aeciospores continue to infect the same host by secondary infection. Telia are formed only after the month of December.

Study of the Sexual Behaviour of the Rust

Recent investigators on rusts have shown the importance of the study of sex in rusts. The discovery of the heterothallic nature of *Puccinia Helianthi* Schw. and *Puccinia graminis* Pers. by Craigie (1931) has attracted the attention of several investigators. By further studies, forms such as *Puccinia coronata* Corda., *P. Sorghi* Schw., *Melampsora* Lini (Pers.) Lév., *Gymnosporangium Juniperi-virginiane* Schw., *Cronartium ribicola* J. C. Fischer, and others have been proved to be heterothallic. Only in *Puccinia Malvacearum* Bert., a doubtful case of homothallism has been reported.

In studying the sporidial infections of the *Trochodium* under study, an attempt was made to investigate the sexual behaviour of the rust. Seedlings of *Argyreia cymosa* grown under rust-free conditions were taken, and sporidial infections were obtained on them by suspending germinating teliospores over the seedlings, as described by Craigie (1931). By this method well isolated monosporidial infections were obtained. The infected plants were covered with bell jars to prevent insect vectors. Care was taken to provide adequate aeration to the plants. Only such leaves in which infected spots were sparsely distributed were chosen for observation. The monosporidial infections remained unchanged without further development, and gradually deteriorated. Later, the infected patch dried up without any aecial development. In another series of experiments, leaves wherein the infected spots were in close proximity (about 1 to 2 mm. apart) were chosen for observation. The nectar exuding from the pycnia was mixed up by gently passing the tip of a camel-hair brush. Aecial development was observed after 16 or 20 days. These experiments indicate that the *Trochodium* on *Argyreia cymosa* is heterothallic, and that the fusions of the nectar of two different strains of pycnia are essential for the development of aecia. Brown (1940) points out that the presence of a well-developed pycnia would usually indicate that the fungus is heterothallic. In the homothallic forms pycnia are either absent or under-developed.

Conclusions

From the preceding investigations it will be manifest that the *Trochodium* on *Argyreia cymosa* and on *Lettsomia elliptica* is an autoecious but heterothallic rust whose spore-forms develop on the host in definite seasons; pycnia in July and August, aecia (both

primary and secondary) in September to December, and telia from January onwards. Dr. B. B. Mundkur of the Imperial Agricultural Research Institute, New Delhi, kindly informed the writer that Butler's specimens in Assam, the Madras Presidency and the Mysore State, were collected during October to December when only aecia are present.

It will be noted that the aeciospores of *Trochodium* on *Argyreia* and *Lettsomia* can cause secondary infections leading to the spread of the rust, and as in *Uromyces Hobsoni* the uredial stage is absent. There were certain other characters likewise in which it resembled *U. Hobsoni*, namely, the occurrence of flask-shaped pycnia with ostiole and paraphyses, cupulate aecia with well-developed peridia, secondary infection by the aeciospores, autoecious life-cycle, and the development of secondary and tertiary sporidia.

It is also clear that the rust differs substantially from the only other species of *Trochodium* so far known, *T. Ipomoeæ* (Thüm.) Syd. in its morphological characters. In naming the fungus, two courses were open to the writer, either to make a new combination using Berkeley and Broome's specific epithet, or erect a new species. If he were to adopt the former course, then the type would be Berkeley and Broome's rust on *Argyreia elliptica* collected at Peradineya, Ceylon, in January 1868. But this type is without the perfect, that is the telial stage, and it is necessary to designate a new type with the three stages (0, I and III) on it. Taking advantage of Art. 57 of the *International Rules of Botanical Nomenclature* which invalidates all names given to imperfect stages, the writer proposes to present this rust as a new species and calls it *Trochodium Sampathense*.

Description of the rust :

Trochodium Sampathense Thirumalachar sp. nov.

Pycnidiis.—Amphigenis, in hospitali textu residens, formā ampullæ simile, cum ostiolā et paraphysi. Aecidiis amphigenis, in foliis fructibusque catervatim singulis centris dispositum, poculo simile, cum peridio. Aecidiosporis rotunda vel polyhydralis, flava, minutis, cum spinis, 20–17 μ diam. Teleutosporis singulis cellis, fulva vel nigra, latitudo maior est quam longitudo, apice curvato, germinis foramine elevato, rugatā, in caveā posita, exosporā costatā costis ex apice radiantibus et ad fundamentum convergentibus ; 26μ, latis ; 20μ, altis ; caule persistente hyalino, modo in loco afficiendi inflato ; sporis post tempus requietis germinantibus. Sporidis postquam, insitu germinaverunt, secundaria sporidia formant.

Hab.—In foliis vivis *Argyreiæ cymosæ* Sweet. Hiriyur, Mysore State, 5-4-1940 (Typus), *Lettsomia elliptica* Wight, Bangalore, 10-5-1940.

Pycnia.—Amphigenous, sunken in the host tissue, flask-shaped with an ostiole and paraphyses. Aecia are amphigenous found on leaves and fruits in concentric patches ; aeciospores are round or

polyhedral, yellow in colour, minutely spinescent, and measure $20 \times 17 \mu$. Telleuto spores are unicellular, chestnut brown to black in colour, broader than long, with a rounded apex, and measure $26 \times 20 \mu$. Germpore is apical, situated in a ridged pit and the exospore is thick with longitudinal striæ which radiate from the apex and converge towards the base. The stalk is persistent, hyaline and inflated only at the place of attachment. Teleutospores germinate after a period of rest. Sporidia germinate *in situ* and form secondary and tertiary sporidia.

In living leaves of *Argyreia cymosa* Sweet. Hiriyur, Mysore State, 5–4–1940 (Type.) and of *Lettsomia elliptica* Wight. Bangalore, 10–5–1940. Type deposited in the Herb. *Crypt. Ind. Orient.* of the Imperial Agricultural Research Institute, New Delhi, and the Imperial Mycological Institute, Kew, England.

Summary

1. *Trochodium Sampathense* found parasitic on the leaves, twigs and fruits of *Argyreia cymosa*, and *Lettsomia elliptica* is a new species and differs from the only other species of *Trochodium* so far known *T. Ipomoeæ* (Thüm.) Syd. in morphological characters.

2. Three spore-forms *viz.*, telia aecia and pycnia are present in the life cycle of the rust, and these develop on the host in definite seasons ; pycnia in July–August, aecia in September-December, and telia from January onwards. *Aecidium Argyreoides* recorded by Butler and Sydow is the aecial stage of *Trochodium*. Uredia are absent.

3. Pycnia are flask-shaped, sunken in the host tissue, with an ostiole and paraphyses. They have been recorded for the first time for the genus *Trochodium*.

4. Aecia are cupulate, with peridia. Aeciospores are round or polyhedral in shape, and they infect the same host forming secondary aecia.

5. Teliospores germinate after a period of rest. Sporidia are uninucleate in early stages, and binucleate after the formation of secondary sporidia. Tertiary sporidia have been observed.

6. Sporidia infect the same host and form pycnia and aecia.

7. Monosporidial infections reveal that the fungus is hetero-thallic.

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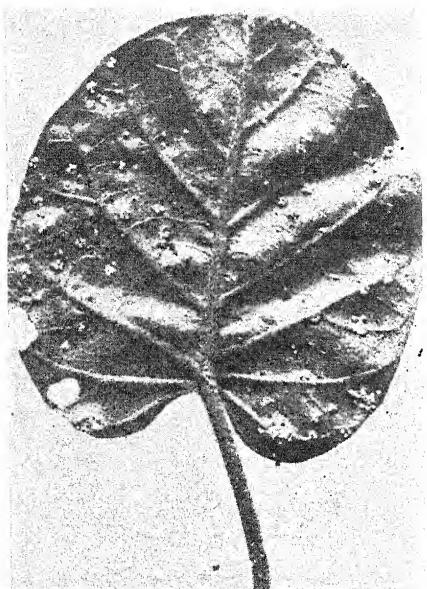
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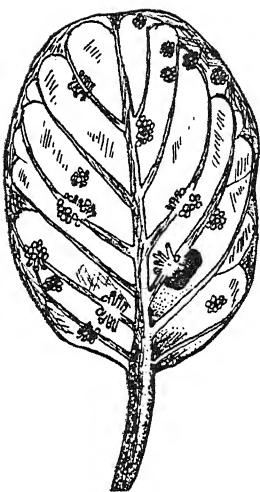
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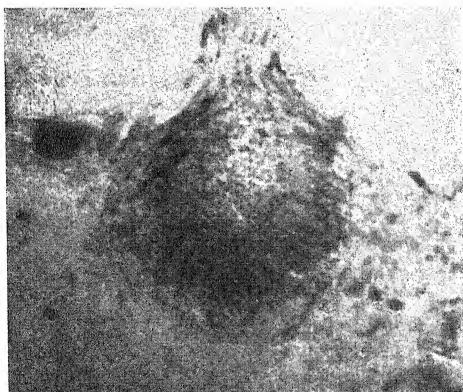
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M. J. THIRUMALACHAR

TROCHODIUM SAMPATHENSE *THIRUMALACHAR SP. NOV.*

GAMETOGENESIS AND EMBRYOGENY IN A FEW MEMBERS OF THE *MELASTOMACEAE*[*]

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(Communicated by M. A. Sampathkumaran)

BENTHAM AND HOOKER (1862–'83), Engler and Gilg (1924), Bessey (1915) and Wettstein (1924) have all placed the family *Melastomaceæ* in the order Myrtales (or Myrtifloræ). But Hutchinson (1926) splits this order into the Lythrales and Myrtales, and places in the latter order the family *Melastomaceæ* along with the Myrtaceæ, Lecythidaceæ, Combretaceæ and Rhizophoraceæ.

Subsequent to the work of Hofmeister (1858), working on the Gametogenesis of *Centradenia*, who reported some interesting features, Mellink (1880), Tassi (1898) and Warming (1898) investigated in detail the embryological features of some members of this family. The latest contribution in this field is by Ruys (1925) and Ziegler (1925), who studied a large number of European species.

The present study on the gametogenesis and embryology in the *Melastomaceæ* was undertaken by the writer in order to find out the morphological details in some of the Indian forms. The plants selected for study are *Leandra cordifolia* Cogn., *Osbeckia cupularis* Don., *O. Wightiana* Benth., *O. hispidissima* Wt., and *Memecylon Heyneanum* Benth.

MATERIALS AND METHODS

The materials for study were collected from the following localities. *Leandra cordifolia* Cogn., a native of South America, was collected in the Government Botanic Gardens, Travancore ; *Osbeckia cupularis* Don., at Kavale in Malabar ; *O. Wightiana* Benth., at Sheratere island, twenty miles from Cochin ; *O. hispidissima* Wt., in marshy places near Balehonnur, Mysore State ; and *Memecylon Heyneanum* Benth., from the Government Botanical Gardens, Bangalore.

The materials were collected on warm and bright sunny days between 11 A.M. and 3 P.M. Various fixatives were tried, of which Bouin's and Nawashin's proved to be the best. To facilitate penetration the hairs on the ovary wall were removed and the materials were kept in a vacuum condition. Dehydration, clearing and embedding were done as usual. In the case of *O. hispidissima* the quick

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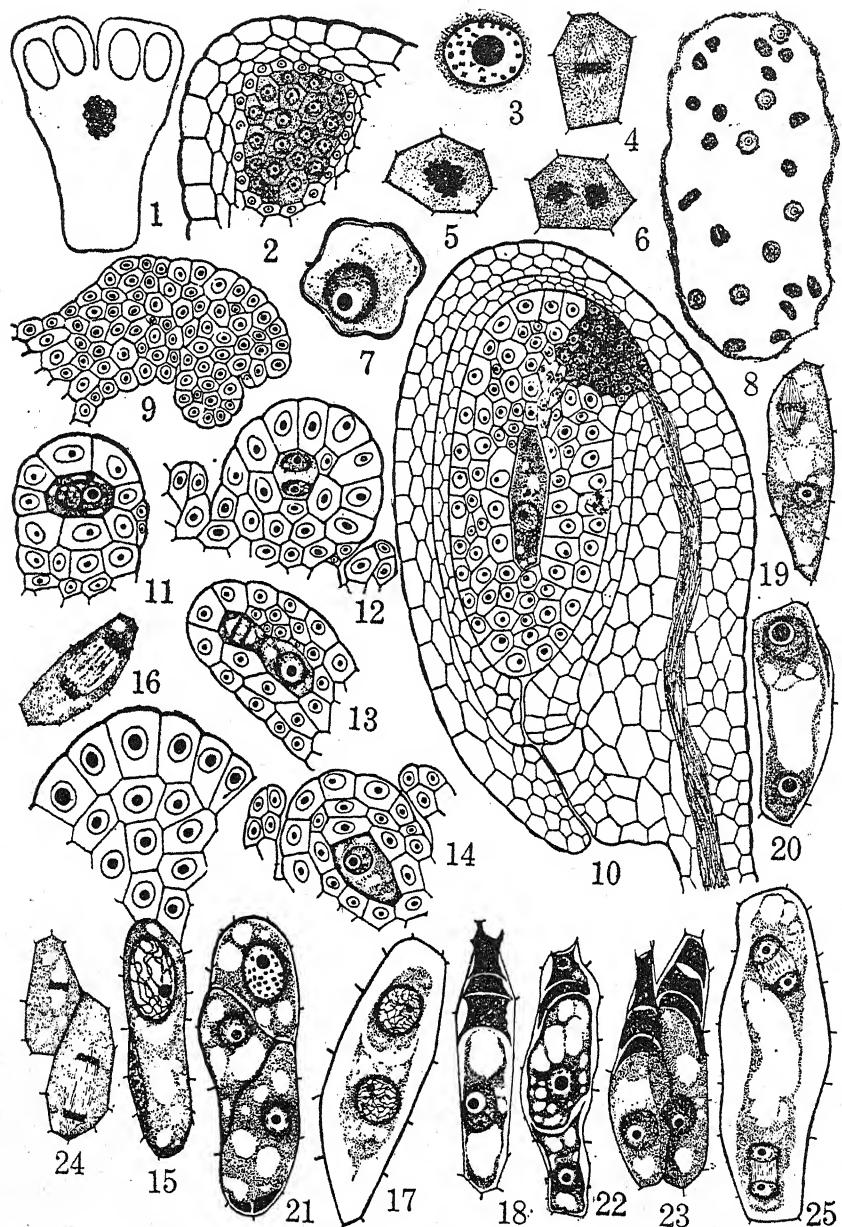
Figs. 1–25.—*Leandra cordifolia* Cogn. Fig. 1. Transverse section of the anther to show the four locular nature (×140). Fig. 2. A portion of the locule of the anther to show the epidermis, two middle layers, tapetum and the sporogenous tissue (× 450). Fig. 3. Pollen mother cell in diakinesis, showing 23 bivalents (× 1800). Fig. 4. Pollen mother cell in

method of infiltration as recommended by Dawson (1922) was tried and was found to be very successful. Sections were cut from 6 to 16 microns in thickness, and stained in Heidenhain's iron-alum hæmatoxylin, with Orange G as counterstain in some cases. For microsporogenesis the slides were destained in a saturated solution of picric acid.

MICROSPORANGIUM AND MICROSPOROGENESIS

The development of the microsporangium has been studied in *Memecylon Heyneanum* and *Leandra cordifolia*.

There are eight stamens in *Memecylon Heyneanum* and ten in *Leandra cordifolia*. The anther shows a four locular nature in cross section (Fig. 1). In the young anther three to four cells are differentiated in the hypodermal layer to form the primary archesporium (Fig. 62). These cells divide periclinally and give rise to the inner sporogenous and the outer primary parietal cells. The latter divide further and give rise to three parietal layers, of which the outermost forms the endothecium and the innermost the tapetum (Fig. 66). The sporogenous cells divide and give rise to the microspore mother cells. The tapetal cells are uninucleate throughout and thus correspond to the first type recognized by Cooper (1933) in his classification of the tapetal cells in Angiosperms. The tapetal cells attain their maximum size at the tetrad stage of the mother cells. They become slightly detached from the middle layer in the case of *Memecylon Heyneanum*. They show rich contents but later disorganise, thus affording nutrition for the developing tetrad of microspores. Further, in the case of *M. Heyneanum* the tapetal cells divide periclinally to form two layers of tapetal cells (Fig. 67).

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metaphase (× 180). Fig. 5. Polar view of the metaphase plate (× 1800). Fig. 6. Two polar metaphase plates in meiotic mitosis showing the parallel disposition of the spindles (× 1800). Fig. 7. Mature uninucleate pollen grain (× 1800). Fig. 8. A portion of the locule of the anther showing the disorganised tapetum and the pollen grains in various stages of degeneration (× 400). Fig. 9. An young anatropus ovule to show the development of the initials of the integuments (× 400). Fig. 10. An anatropous ovule showing the vascular strand, the hypostase, the megaspore mother cell, the two integuments and the zigzag micropyle (× 900). Fig. 11. The primary archesporium (× 900). Fig. 12. The archesporium in late telophase division (× 900). Fig. 13. Megaspore mother cell and the primary parietal cell in late anaphase division (×900). Fig. 14. Two parietal cells and the megaspore mother cell (× 900). Fig. 15. Megaspore mother cell in open spireme stage (× 900). Fig. 16. Megaspore mother cell in late anaphase stage (× 900). Fig. 17. Dyad (× 900). Fig. 18. Linear tetrad showing the degenerating micropylar megaspores and the enlarging chalazal megaspore (× 900). Fig. 19. Two-nucleate embryo-sac, where the micropylar nucleus is in metaphase division (× 900). Fig. 20. Two-nucleate stage (× 900). Fig. 21. Three megaspore mother cells out of which one is in diakinesis (× 900). Fig. 22. Linear tetrad where the third and chalazal megaspore show signs of enlargement (× 900). Fig. 23. Double linear tetrad (× 900). Fig. 24. Two megaspore mother cells in division, one in metaphase and the other in anaphase (× 900). Fig. 25. The formation of the four-nucleate embryo-sac from two-nucleate embryo-sac (× 900).
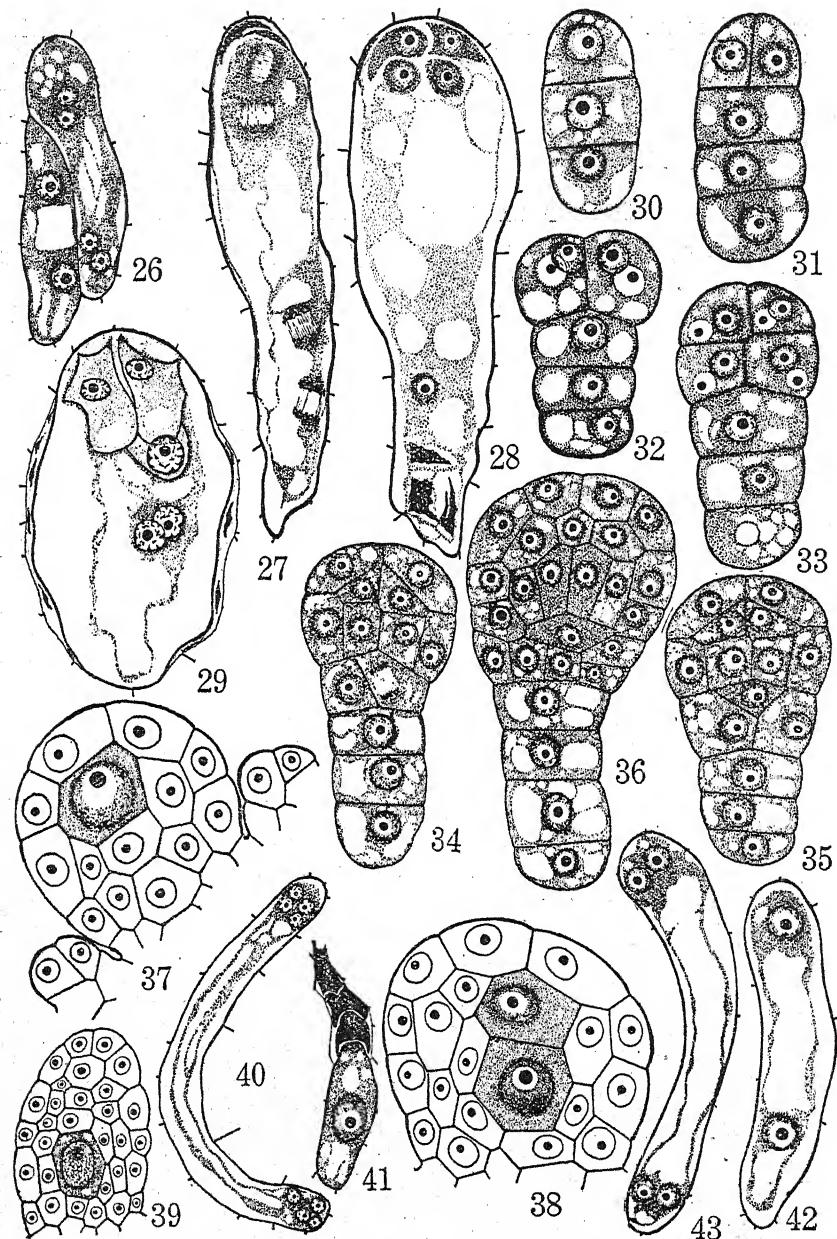
Figs. 26–43.—*Leandra cordifolia* Cogn.　Fig. 26. Double embryo-sacs—one in the two-nucleate stage and the other in the four-nucleate stage (× 900). Fig. 27.　The formation of the eight-nucleate embryo-sac from the four-nucleate stage (× 900).　Fig. 28.　An eight-nucleate embryo-sac showing

The nucleus of the pollen mother cells passes through the usual meiotic divisions (Figs. 3, 4, 5, 63, 69 and 73). The nucleolus may or may not persist till late diakinesis. During the first metaphase there are seven chromosomes in *M. Heyneanum* (Fig. 63), and twenty-three in *L. cordifolia* (Fig. 3). The bivalents are drawn towards the poles. The telophase chromosomes form a compact group and the spindle attains its greatest length. A distinct nuclear membrane is formed around each telophase set of chromosomes but no wall is formed after the first division; on the completion of the second division the tetrad separates by cleavage furrows (Fig. 65).

The mature pollen grains have a thick exine and a thin intine. At the stage of shedding they are binucleate in the case of *M. Heyneanum* (Fig. 71) and uninucleate in *L. cordifolia* (Fig. 7).

In the case of *L. cordifolia* there are only four to six fully developed pollen grains in each anther locule (Fig. 8). This is due to pollen sterility, as a large number of pollen grains was seen in various stages of degeneration. In *M. Heyneanum* all the microspore mother cells develop into pollen grains without degenerating.

In the mature condition the anther shows an outermost layer the epidermis; next to this an endothecium, which is greatly developed in *M. Heyneanum* with the characteristic thickenings; a single middle layer; and lastly the tapetum (Fig. 66).

GERMINATION OF POLLEN GRAINS

In the case of *M. Heyneanum* pollen grains were germinated on slides on drops of sucrose solution.

The first sign of germination of the pollen grain is a slight swelling probably due to the absorption of sugar solution, when the three germ pores are clearly seen. The intine bulges out as a small pellicle through each of the germ pores, further development of the tube being restricted to one of the pores alone (Figs. 70, 71 and 72).

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the early degeneration of the three antipodals before the organisation of the egg apparatus and micropylar polar (× 900). Fig. 29. Mature embryosac showing the egg, the hooked synergids and the polars (× 900). Fig. 30. Three-celled proembryo (× 900). Fig. 31. Two-celled embryo (× 900). Fig. 32. Four-celled embryo (× 900). Fig. 33. Eight-celled embryo (× 900). Fig. 34. Formation of the second oblique wall in the penultimate cell of the embryo (× 900). Fig. 35. The formation of the triangular hypophysis (× 900). Fig. 36. A late embryo with four suspensor cells (× 900). *Osbeckia cupularis*, Don. Fig. 37. The primary archesporium (× 1800). Fig. 38. The archesporium having divided periclinally to form the primary parietal cell and the megaspore mother cell (× 1800). Fig. 39. Megaspore mother cell and three parietal cells (× 900). Fig. 40. An young eight-nucleate embryo-sac (× 900). Fig. 41. Linear tetrad in which the upper three megaspores have degenerated (× 900). Fig. 42. Two-nucleate embryo-sac (× 900). Fig. 43. Four-nucleate embryo-sac (× 900).

Megasporangium and Megasporogenesis

The ovary is semi-inferior with a single short style and is covered by a persistent calyx. It is setose at the apex in *O. cupularis* and fringed with silky hairs or bristles at the apex in *O. Wightiana* and *O. hispidissima*. It is small and has a filiform style in *M. Heyneanum*.

The ovary is pentalocular in *L. cordifolia* and *O. Wightiana*, quadrilocular in *O. cupularis* and *O. hispidissima* (Fig. 82), and monolocular in *M. Heyneanum* (Figs. 68 and 74). The ovules are indefinite in number and arise on the slightly projecting axile placenta in all the plants, except in *M. Heyneanum* where the ovary is monolocular containing nine ovules arranged in a whorl on a free central placenta (Fig. 74). The ovules are anatropous and bitegumentary, their micropyles pointing towards the placental ridge (Figs. 10 and 75).

Each ovule takes its origin as a nucellar primordium and soon grows in size. The gradual development of the anatropous condition could be traced in all its stages. First, the inner integument is formed as an annular growth (Fig. 9). Very soon the outer integument is also formed and simultaneously with this the ovule begins to curve. Now the two integuments grow on either side of the nucellus and finally grow even beyond it. The micropyle is thus formed by both the integuments. The portion of the micropyle formed by the outer integument is not in line with that formed by the inner integument. Thus the micropyle appears to be somewhat zigzag (Figs. 10 and 75). A similar condition is seen in the Lythraceæ (Joshi and Venkateswarlu, 1925, 1936) and in the Sonneratiaceæ (Venkateswarlu, 1937). In all the plants studied both the integuments are two cells thick and consist of uniform cells, except in *L. cordifolia* where the outer integument is made up of three layers of cells (Fig. 10). At first both the integuments are two cells thick throughout their length. The inner integument remains so even in the mature ovule except near the micropyle, where some of the cells of its outer layer divide once and the integument becomes three cells thick. In the micropylar region the cells of the outer integument also divide and form three to five layers of cells. In the Punicaceæ, Combretaceæ, and in one member of the Lythraceæ, *Cuphea* (Mauritzon, 1934), the outer integument is many-layered; in the Lecythidaceæ and Rhizophoraceæ both the integuments are made up of many layers of cells (*cf.* Mauritzon, 1939).

Simultaneous with the growth of the inner integument a single large hypodermal archesporial cell is differentiated as reported by Ruys and Zeigler (1925). In *O. Wightiana* the archesporium arises after the formation of the integuments (Fig. 52), while in the others the archesporium can be recognised even earlier at the time of the origin of the two integuments (Figs. 12, 37 and 76).

In all the plants studied the usual condition is the development of a single archesporial cell. In *L. cordifolia*, however, since double

megaspore mother cells, double linear tetrads, and double embryo-sacs were occasionally met with, it is probable that a multiple archesporium may also be present (Figs. 21, 23, 24 and 26). In the Lythraceæ (Joshi and Venkateswarlu, 1935, 1936) and in the Sonneratiaceæ (Venkateswarlu, 1937) the multiple archesporium appears to be the usual condition. Among the allied families, according to Schnarf (1929), a multiple archesporium has been described in the Rhizophoraceæ by Karsten (1891), Nyssaceæ by Horne, and in a number of Onagraceæ by Michealis, Schwemmle, Täckholm and Häkansson (Joshi and Venkateswarlu, 1936). Recently Tiwary and Rao (1934), have noted the occasional occurrence of a multicellular archesporium in *Callistemon linearis* (Myrtaceæ).

The archesporium can be recognised by its rich cytoplasm and prominent nucleus. It divides by a periclinal wall to form the primary parietal cell and the megaspore mother cell (Figs. 12 and 38). The presence of a parietal cell is noted in all the families of the Myrtifloreæ (*cf.* Mauritzon, 1939), except the Lecythidaceæ (*cf.* Mauritzon, 1939). In all the plants studied by the writer the primary parietal cell undergoes further periclinal divisions and gives rise to a large parietal tissue (Figs. 13, 39, 53, 77 and 83) in which the epidermal cells do not participate.

As a result of the formation of parietal cells, the megaspore mother cell becomes deep seated in the tissue of the nucellus. At the region where the chalazal vascular strand enters the ovule, a group of nucellar cells with rich cytoplasmic contents can be differentiated (Figs. 10 and 61); these cells resemble the hypostase tissue which is reported in the allied family, Onagraceæ (Johanson, 1928), probably nutritive in function. The formation of the hypostase-like tissue takes place at different stages of development of the ovule. In *L. cordifolia* and *O. Wightiana* they are seen to develop even at the megaspore mother cell stage whereas in *O. cupularis* and *O. hispidissima* their development is belated; and in *M. Heyneanum* such a type of tissue is absent. The occurrence of the type of chalazal strand of elongated cells as reported in Punicaceæ (1939), Myrtaceæ (1939), Lythraceæ (1935, 1936) and Sonneratiaceæ (1937), is absent in the Melastomaceæ.

The nucellus is nearly straight except for a small curvature at the chalaza. There are two to three layers of nucellar cells at the sides of the embryo-sac in all the plants except *M. Heyneanum* where there are three to four layers of cells. The enlargement of the embryo-sac during its development is accompanied by the disorganisation of the nucellus with the result that only a single layer of parietal tissue is left above the embryo-sac. But in *M. Heyneanum* two such layers are present.

In the course of its development, the megaspore mother cell enlarges in size. It undergoes the usual two divisions (Figs. 15, 16, 39, 53, 77 and 83), and forms the linear tetrad of megaspores (Figs. 18, 41, 56, 78 and 84). In all the plants studied it is usually the chalazal megaspore that develops further.
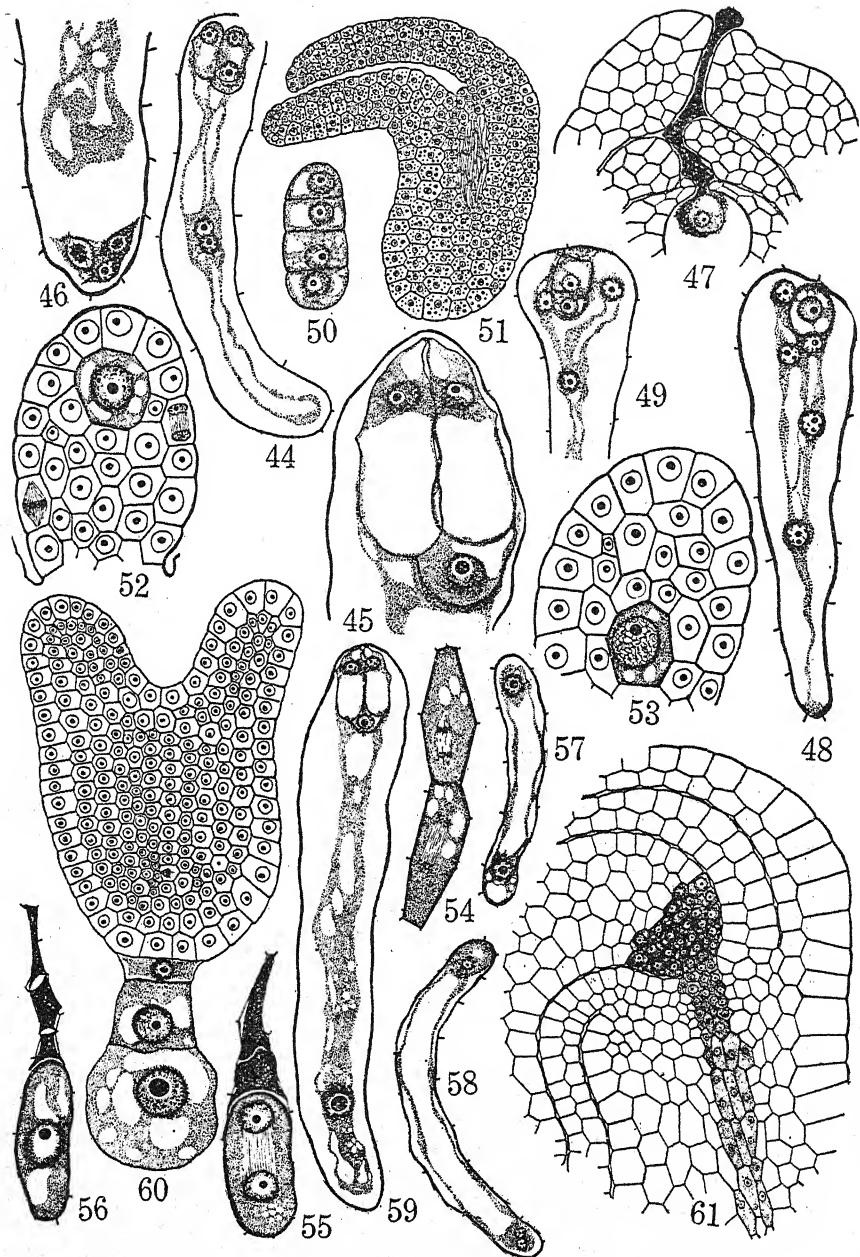
Figs. 44–61.—*Osbeckia cupularis*, Don.   Fig. 44.   Mature embryo-sac
(× 450). Fig. 46. The chalazal portion of the embryo-sac enlarged to show
the degenerating antipodals (× 900). Fig. 47. Entry of the pollen tube
through the micropyle of the ovule (× 180). Fig. 48. Fertilized egg and

Sometimes the third megaspore was also seen enlarging with the usual chalazal one in *L. cordifolia* (Fig. 22), and in *O. hispidissima* both the micropylar and chalazal megaspores were found developing together (Figs. 86 and 87). The significance of these features will be pointed out at the end of this paper.

During the development of the chalazal megaspore two vacuoles, one at each pole begin to be formed. Later, when the two-nucleate embryo-sac develops, a central vacuole also appears. At this stage, only one of the polar vacuoles, usually the chalazal one, persists and may be seen even when the four-nucleate embryo-sac is formed (Figs. 42, 43, 57, 58, 79, 80 and 88). A similar condition has been reported in the Lythraceæ (Joshi and Venkateswarlu, 1935-1936). With the exception of *L. cordifolia* and *M. Heyneanum* the embryo-sac is slightly bent at the four-nucleate stage in all the genera studied (Figs. 40, 58 and 89).

In all the five plants the antipodals degenerate early as previously noted by Ruys (1924), Ziegler (1925) and Mellink (1880) in this family (Figs. 28 and 46).

The mature embryo-sac is long and narrow in all the plants except *L. cordifolia* in which it is oval (Figs. 29, 44, 59, 81 and 90). In *M. Heyneanum* the mature embryo-sac is very long, bent and narrow at the central region (Fig. 81). The polar nuclei which are at first in either pole of the embryo-sac, meet in the centre.

The egg apparatus consists of the usual two synergids and the egg. The tips of the synergids are slightly pointed and they begin to develop characteristic hooks from an early stage. Similar instances of hooked synergids have been reported amongst closely allied groups in the Lythraceæ (Mauritzon, 1934 ; Joshi and Venkateswarlu, 1935–1936) and the Myrtaceæ (*Callistemon linearis*, Tiwary and Rao, 1934).

In *O. Wightiana* the synergids show apical vacuoles (Fig. 45) similar to those in the Lythraceæ (Joshi and Venkateswarlu, 1935–1936). In discussing the importance of these vacuoles Joshi and Venkateswarlu (1936) state, " that they take the place of the

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free endosperm nuclei ($\times$ 450). Fig. 49. Two-celled proembryo and free endosperm nuclei ($\times$ 450). Fig. 50. Four-celled proembryo ($\times$ 900). Fig. 51. A very late embryo showing the two cotyledons and the stem tip in between, the cells of the embryo contain deeply staining particles ($\times$ 400). *Osbeckia wightiana*, Benth Fig. 52. The primary archesporium ($\times$ 900). Fig. 53. Megaspore mother cell and parietal cells ($\times$ 900). Fig. 54. Division of Dyads to form the linear tetrad ($\times$ 900). Fig. 55. Degeneration of megaspores in the linear tetrad ($\times$ 900). Fig. 56. Linear tetrad showing the enlarging chalazal megaspore ($\times$ 900). Fig. 57. Two-nucleate embryo-sac ($\times$ 450). Fig. 58. Four-nucleate embryo-sac ($\times$ 80). Fig. 59. Mature embryo-sac showing the egg apparatus and the secondary nucleus ($\times$ 400). Fig. 45. The egg apparatus enlarged to show the apical vacuoles in the synergids ($\times$ 900). Fig. 60. A late embryo with the basal cell enlarged ($\times$ 180). Fig. 61. The chalazal portion of the ovule enlarged to show the nutritive tissue resembling the hypostase ($\times$ 180).
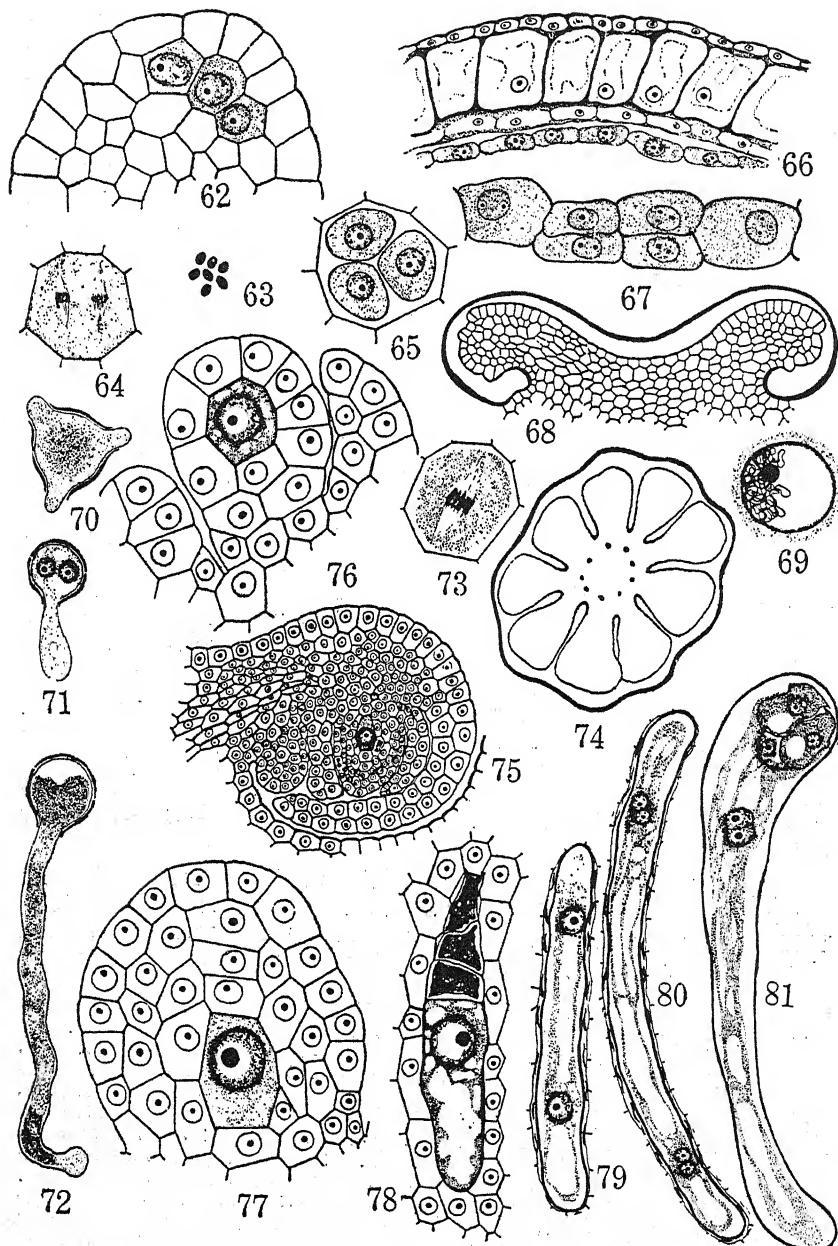
Figs. 62–81.—*Memecylon Heyneanum*, Benth. Fig. 62. A very early stage showing the initiation of the archesporium (× 900). Fig. 63. Metaphase plate showing seven bivalents (× 1800). Fig. 64. Parallel disposition of the spindles after the second division (× 1350). Fig. 65. Microspores within the microspore mother cell wall (× 1350). Fig. 66. A portion

'filiform apparatus' seen in other plants and serve probably the same function *viz.*, that of secreting chemotropically active substances which guide the pollen tube."

Double embryo-sacs have been seen in *L. cordifolia*. It is likely that they are derived from two megaspores belonging to different linear tetrads, for cases have been met with showing two megaspore mother cells, sometimes also three (Fig. 21), developing further into linear tetrads lying side by side (Figs. 23, 24 and 26). The development of the double embryo-sacs proceeds up to the four-nucleate stage after which one degenerates (Fig. 26).

FERTILIZATION

During the present study the entire process of fertilization could not be followed in detail in all the plants, but the entry of the pollen tube was observed in *O. cupularis* (Fig. 47). The pollen tube enters through the micropyle and crushes one of the synergids during its passage into the embryo-sac.

ENDOSPERM

A free nuclear endosperm has been reported for a large number of plants of this family (Ruys, 1924 and Ziegler, 1925). The primary endosperm nucleus divides before the first division of the fertilized egg and gives rise to free endosperm nuclei. The disposition of these nuclei is interesting ; there is a definite accumulation of these nuclei in the chalazal and micropylar regions of the embryo-sac (Figs. 48, 97 and 98) noted by Joshi and Venkateswarlu (1935–1936) in the Lythraceæ. No walls are formed between these nuclei even in later stages ; the nuclear endosperm is later used up as such by the developing embryo in the seed, which consequently becomes non-endospermic at maturity.

EMBRYOGENY

In general the development of the embryo agrees with the *Capsella*-type except in small details. In *L. cordifolia* the first division is transverse and a two-celled proembryo is formed. Later

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of the wall of the mature anther enlarged to show the epidermis, endothecium, middle layer and the tapetum ($\times$ 180). Fig. 67. A portion of the tapetum enlarged ($\times$ 900). Fig. 68. Longitudinal section of the monolocular ovary showing the two ovules attached on a free central placenta ($\times$ 180). Fig. 69. Pollen mother cell in synizesis ($\times$ 1350). Fig. 70. Early stage in the germination of the pollen grain showing the three protoplasmic pellicles coming through the three germ pores ($\times$ 900). Fig. 71. Germination of the pollen tube through one of the germ pores ($\times$ 900). Fig. 72. A well-developed pollen tube ($\times$ 900). Fig. 73. Pollen mother cell in metaphase ($\times$ 1350). Fig. 74. Transverse section of the monolocular ovary showing nine ovules arranged on a free central placenta ($\times$ 16). Fig. 75. An anatropous ovule showing the megaspore mother cell, four parietal cells, two integuments and the zigzag micropyle ($\times$ 80). Fig. 76. The archesporium ($\times$ 900). Fig. 77. Megaspore mother cell and three parietal cells ($\times$ 900). Fig. 78. Linear tetrad ($\times$ 450). Fig. 79. Two-nucleate embryo-sac ($\times$ 180). Fig. 80. Four-nucleate embryo-sac ($\times$ 400). Fig. 81. Mature embryo-sac ($\times$ 400).
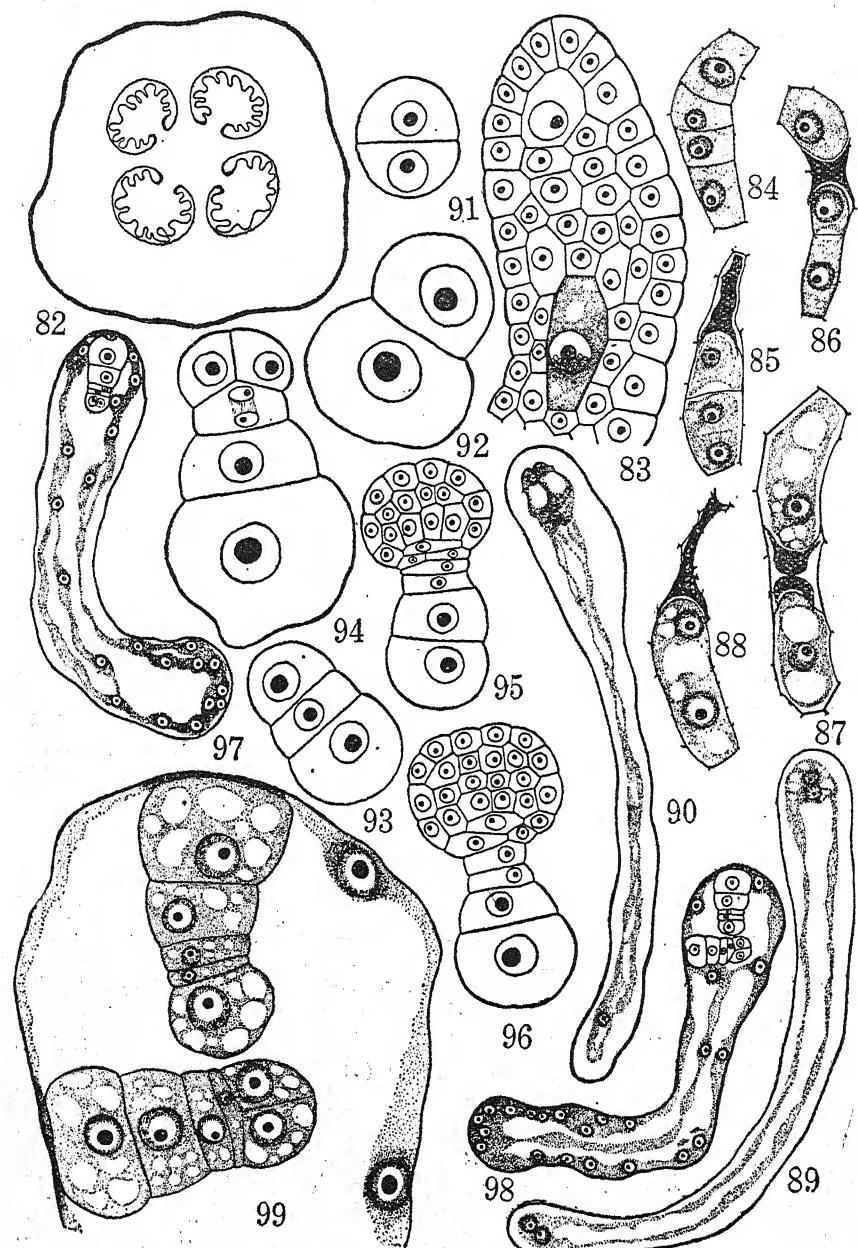
Figs. 82–99.—*Osbeckia hispidissima* Wt. Fig. 82. Transverse section of the ovary to show the four locular nature and the nucellar primordia arising on the axile projecting placenta (× 16). Fig. 83. Megaspore mother cell in synizesis and five parietal cells (× 900). Fig. 84. Linear

the proembryo becomes filamentous (Figs. 30, 31). The terminal cell of this proembryo begins to assume a more or less spherical shape and divides into quadrants and then into octants. The four upper octants form the stem and cotyledons and the four basal ones the hypocotyl except its tip. In the octant stage the dermatogen is cut off by periclinal walls and the periblem and plerome are formed afterwards (Figs. 31, 32, 33, 34, 35 and 36).

Just when the dermatogen is differentiated, two intersecting oblique walls are laid in the penultimate cell of the proembryo (Figs. 34 and 35). A small triangular cell is formed and this is called the hypophysis (Fig. 35) which differs from the usual type seen in *Capsella*. The hypophysis then divides transversely to form two cells, the inner of which completes the periblem and the outer the dermatogen. The suspensor is three celled in the early stages, but later becomes four celled (Fig. 36).

In *O. cupularis* the cells of the old embryo contain large quantities of deeply staining reserve materials (Figs. 48, 49, 50 and 51).

In *O. Wightiana* the basal cell of the suspensor enlarges in size (Fig. 60).

In *O. hispidissima*, the fertilized egg divides and forms a two-celled proembryo. Of these the cell towards the micropyle does not divide further (Figs. 91, 92). The development of embryo and other suspensor cells is due to the activity of the other cell which forms a filament. The terminal cell of this filament forms the embryo. In later stages further additions to the suspensor are made by intercalary divisions of the cells of the suspensor (Figs. 93, 94, 95 and 96).

In *O. hispidissima*, one interesting case showing the development of two embryos in the same embryo-sac was met with during this investigation. In this the second embryo was disposed at right angles to the usual one at the micropylar end (Figs. 98, 99). In the absence of other stages the exact mode of origin of the second embryo cannot be dealt with now. Its orientation, however, suggests that it arises as a result of nucellar budding.

It is interesting to note that polyembryony in the order Myrtifloræ has so far been known only in the Myrtaceæ (Tiwary, 1926).

Further work on the Melastomaceæ may yield interesting results.

tetrad of megaspores before the degeneration of the upper megaspores ($\times$ 900). Fig. 85. Degeneration of the first megaspore in the linear tetrad ($\times$ 900). Figs. 86–87. Linear tetrad where the micropylar and chalazal megaspores show signs of enlargement ($\times$ 900). Fig. 88. Two-nucleate embryo-sac with the three persisting degenerated micropylar megaspores ($\times$ 900). Fig. 89. Four-nucleate embryo-sac ($\times$ 900). Fig. 90. Mature embryo-sac ($\times$ 180). Fig. 91. Two-celled proembryo ($\times$ 900). Fig. 92. Two-celled proembryo in which the basal cell has enlarged ($\times$ 900). Fig. 93. Three-celled proembryo ($\times$ 900). Fig. 94. Two-celled embryo ($\times$ 900). Figs. 95–96. Two stages of late embryos ($\times$ 900). Fig. 97. Embryo with accumulations of free endosperm nuclei in the micropylar and chalazal regions of the embryo-sac ($\times$ 80). Figs. 98–99. Two embryos in the same embryo-sac ($\times$ 900).

DISCUSSION AND CONCLUSIONS

The position of the family according to the different systems of classification has already been pointed out in the introductory part of this paper. The present study shows that there is very little justification for splitting the order Myrtifloræ of Engler and Prantl into the two orders Lythrales and Myrtales as proposed by Hutchinson.

In the structure of the integuments and in the formation of the micropyle, the condition met with in the Melastomaceæ recalls very strongly the features in the Lythraceæ (*cf.* Mauritzon, Joshi and Venkateswarlu, 1935–1936). But the chalazal strand which is very characteristically seen in the Lythraceæ is conspicuously absent in the Melastomaceæ. On the other hand, the chalazal region of the ovule is here characterised by the presence of a large nutritive tissue resembling the hypostase which is a distinctive feature of the Onagraceæ. In the formation of the parietal cell, as well as in the development of a large parietal tissue, the families Lythraceæ and Melastomaceæ appear to agree closely. The archesporium is made up of a single cell, but from indirect evidence of double linear tetrads and double embryo-sacs, it is surmised that it may occasionally consist of more cells. A multiple archesporium is described in the case of Lythraceæ (Joshi and Venkateswarlu, 1935–36).

Attention may be directed to the fact that occasionally a second megaspore may also survive for a period, along with the usual chalazal megaspore. The enlargement of the first and third megaspores in addition to the chalazal megaspore has been observed in *L. cordifolia* and *O. hispidissima*. Similar instances have been recorded by Joshi and Venkateswarlu in *Lawsonia inermis*; and in *Miconia* and in *Memecylon ensiformis* by Ruys. In *Miconia* and *Memecylon* Ruys noticed that the development of the micropylar megaspore was the usual feature. It may be mentioned in this connection that in the case of the Onagraceæ the micropylar megaspore is invariably the surviving one. It is probable, that the behaviour of the megaspores in the Melastomaceæ offers some indication of the gradual domination attained by the micropylar megaspore in reaching the condition seen in the Onagraceæ.

The development of the embryo-sac indicates some important features which seem to throw some light on the probable affinities of the Melastomaceæ. The development of a chalazal vacuole in the young embryo-sac bears a striking resemblance to a similar condition found in the Lythraceæ. The synergids are not only hooked as described in the case of Lythraceæ (1936), but appear to be sometimes characterised by the formation of apical vacuoles. Further, the early degeneration of the antipodals seems to be a very characteristic feature. Joshi and Venkateswarlu state that, " These points....have been regarded by Tischler and Mauritzon to indicate that the embryo-sac of the Lythraceæ forms phylogenetically an intermediate stage between the four-nucleate embryo-sac of the Onagraceæ and the normal eight-nucleate embryo-sac,"

The endosperm develops according to the free nuclear type in the other families of this order. The accumulation of endosperm nuclei which is seen in the Lythraceæ is also met with in Melastomaceæ. The one important difference between the endosperm in the Melastomaceæ and that in the Lythraceæ is that in the former walls are not seen at any stage during the development of the seed, whereas in the latter, a loose endosperm tissue is present. In this connection it may be noted that among the Oenotheraceæ sometimes as in *Lopezia coronata* (Täckholm), there is no wall formation.

The development of the embryo is similar to that in the Lythraceæ and follows the *Capsella*-type except for certain minor details.

Lastly regarding Polyembryony it may be mentioned that Myrtaceæ is the only other family of the order Myrtifloraæ in which the development of additional embryos has so far been reported.

Summing up the embryological features of the Melastomaceæ and comparing them with those of the Lythraceæ, there seems to be hardly any ground for splitting the cohort Myrtifloræ of Engler and Gilg (1924) and others into the two orders Myrtales and Lythrales as suggested by Hutchinson (1926). Joshi (1939) has already remarked that the Lythraceæ and the allied families appear to be so closely related to one another that it is better for the present to keep the several families of the Lythrales (Hutchinson) along with those of the Myrtales (Hutchinson).

SUMMARY

1. A detailed account of the gametogenesis and embryogeny of some members of the *Melastomaceæ, viz., Leandra cordifolia* Cogn., *Osbeckia cupularis* Don., *O. Wightiana* Benth., *O. hispidissima* Wt., and *Memecylon Heyneanum* Benth., is given in the paper.

2. The chromosome numbers for *M. Heyneanum* and *L. cordifolia* have been determined for the first time. They are seven and twenty-three respectively.

3. The ovules are bitegumentary and the outline of the micropyle is somewhat zigzag.

4. Double embryo-sacs have been noted in *L. cordifolia* thereby suggesting the occurrence of a multiple archesporium. However, occurrence of a single archesporium is the usual condition in this family.

5. The hypodermal archesporium cuts off a parietal cell which next gives rise to a parietal tissue.

6. Variations are seen in the order of degeneration of the megaspores in the linear tetrad.

7. The chalazal megaspore usually enlarges and develops into the embryo-sac, but in *L. cordifolia* and *O. hispidissima* variations are met with and more than one megaspore may enlarge for a time.

8. Development of the embryo-sac conforms to the normal-type. The antipodals degenerate early in all the forms; in *O. cupularis*, they persist till the egg apparatus is organised:

9. The synergids are hooked. An apical vacuole is also present in *O. Wightiana.*

10. Entry of the pollen tube through the micropyle has been observed in *O. cupularis.*

11. The endosperm is nuclear. Accumulation of endosperm nuclei in the chalazal and micropylar regions takes place during development.

12. Development of the embryo proceeds according to the *Capsella*-type.

13. A new type of development of the hypophysis is observed in *L. cordifolia.* A triangular cell is cut off and this functions as hypophysis.

14. Two embryos in the same embryo-sac have been noted in *O. hispidissima.*

ACKNOWLEDGMENTS

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THE IDENTITY OF PUNARNABA*

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(Communicated by G. P. Majumdar)

There is a state of confusion as to the real identity of the Ayurvedic drug ' Punarnaba' particularly Swet Punarnaba, so widely reputed as a specific for dropsy, beriberi, ascites and the allied diseases, hence also goes by the name of " Sothaghni " in Sanskrit. In the local markets in Bengal one† *Trianthema Portulacastrum* Linn., a plant of the Family *Ficoideac* is extensively sold by the herbalists. This plant is prescribed by the Kavirajes as Swet Punarnaba. But in almost all works on Indian Pharmacopea and Medicinal Plant including, Col. Chopra's Indigenous Drugs of India, Kirtikar and Bose's publication of Indian Medicinal Plants, Dymock's Pharmacographia indica, Watt's Dictionary of the Economic Products of India, Anislie, Bently and Trimen and others, *Boerhaavia* (Fam. Nyctaginaceæ) has been identified with Punarnaba. *Boerhaavia repens* Linn. (*B. diffusa* Linn.) has been described by many as the Punarnaba of Ayurveda, placing in support the explanation that there are two varieties of this species, one bearing white flower (Swet Punarnaba) and the other with red flower (Rakta Punarnaba). But this being an indigenous medicinal plant originally described in Sanskirt, one must give prominence on the Ayurvedic description of the drug in order to search for the real plant. In the original description of the plant as given in *Banausadhi Darpan* it is mentioned along with other characters that " Swet Punarnaba " bears white flowers, but unfortunately however, none of the species of *Boerhaavia* occurring in India have been mentioned in any book on Indian Systematic Botany to possess white flowers, they are either pinkish or reddish in colour, hence the confusion. *Trianthema Portulacastrum* Linn. may therefore be the real " Swet Punarnaba " as its characters agree with the Sanskirt description and moreover it is used as such throughout India by the Ayurvedic practitioners and is known from time immemorial as the " Swet Punarnaba " in every Hindu house of India. It has, therefore, become imperative to make a side by side chemical study of the two plants in order to find out the active alkaloidal principle " Punarnavine ". It may be mentioned here with regret that sometimes it has so happened that *Trianthema* has been chemically analysed under the wrong name of *Boerhaavia* with a misconceived idea derived from the

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† *Trianthema Portulacastum* Linn. = *Trianthema monogyna* Linn.

literature that *Boerhaavia diffusa* is the real Swet Punarnaba of commerce and hence often the scientific identification of the drug is considered unnecessary. It is interesting to note that a herbalist in Calcutta will always supply *Trianthema* instead of *Boerhaavia* when asked for Punarnaba. Samples of Punarnaba have been collected from several well-known drug manufacturing firms of Calcutta, and on botanical identification they have been found to be *Trianthema Portulacastrum*. The author has also collected genuine specimens of both the red and white varieties of *Trianthema Portulacastrum* and of *Boerhaavia repens* and their chemical and pharmacological investigations have been kindly taken up at the Calcutta School of Tropical Medicine. The research results of the analyses has been published in the *Indian Journal of Medical Research* by Col. R. N. Chopra, N. R. Chatterjee, and S. Ghosh, 2nd October, 1940.

For convenience of the readers in general, an English translation of the original Sanskrit description of the plant together with generic and specific descriptions of *Boerhaavia* and *Trianthema* is given below to facilitate the real diagnosis of these plants.

TRANSLATION OF SANSKRIT DESCRIPTION

White Punarnaba, diffuse annual with branches and stems. It germinates at the first shower of rain, grows and is adorned with flowers and fruits during the rainy season. Therefore it is difficult to procure it in other seasons. If not attacked by other pests it may grow up to 3 or 4 yards. The leaves of the white Punarnaba is almost round, soft and fleshy. The tender branches are covered with short hairs. Its flowers are white. The seeds are like those of Amarantus. The "Rakta Punarnaba" does not dry or die up after the fruits are ripe. Even though the stem may dry up the root remains under the soil and gives rise to new plants at the advent of rains. Therefore the real name of Punarnaba is justified by Rakta Punarnaba. Its leaves, stem and flowers are red. Its leaves are not so thick as those of Swet Punarnaba, not round rather oblong.

GENERIC CHARACTERS OF BOERHAAVIA LINN.
(FAM. NYCTAGINACEÆ)

Erect or diffuse often divaricately branched herbs. Leaves opposite, often in unequal pairs ; flowers small reddish or pinkish, paniculate, umbellate or subcapitate articulated in the pedicel ; bracteoles small, often deciduous, rarely whorled or involucrate. Perianth tube long or short, ovoid below narrowed above the ovary; limb funnel-shaped with 5-lobed margin, the lobes plicate; stamens 1 or 2-5, connate below, exerted ; filaments capillary, unequal ; ovary oblique, stipitate ; ovule erect ; stigma peltate. Fruit enclosed in the ovoid turbinate or clavate, obtuse of truncate perianth tube, round, 5-ribbed or 5-angled, viscidly glandular ; seeds with adherent testa ; embryo hooked ; cotyledons thin, broad ; the outer larger, enclosing a soft scanty albumen.

Boerhaavia Repens Linn. (Specific Description)

A diffuse herb ; root large, fusiform ; stem prostrate or ascending, reaching 2–3 ft. in length, divaricately branched, slender, cylindric, thickened at the nodes, minutely pubescent or nearly glabrous, often purplish. Leaves at each node in unequal pairs, the larger 1–1½", the smaller ½–¾" long, both nearly as broad as long, broadly ovate or suborbiculate, rounded at the apex, green and glabrous above, usually white minute scales beneath, the margins entire, often coloured pink, somewhat undulate, base rounded or subcordate, petiole nearly as long as the blade, slender. Flowers very small, shortly stalked or nearly sessile, 4–10 together in small umbells, arranged in long stalked corymbose, axillary or terminal panicles, bracteoles small, lanceolate, acute. Perianth ⅛" long, ovarial part of the tube 1/20" long, contracted above the ovary, glandular viscid ; limb funnel-shaped, dark pink, with 5 narrow vertical bands outside. Stamens 2–3 slightly exerted. Fruit ⅛" long, clavate rounded, broadly and bluntly 5–ribbed, very glandular (Fig. 1).

Generic Character of Trianthema (Fam. Ficoidæ)

Diffuse prostrate, branched herbs, glabrous, pubescent or papillose, leaves petiolate opposite, unequal, linear, ovate or obovate, quite entire ; stipuleso, but the petiole dilated with membranous stipuli form margins.   Flowers axillary, sessile or peduncled, solitary, cymose or panicled. Calyx tube short or long ; lobes 5 coloured within mucronate at the back. Petioles O. Stamens 5-10 or many, inserted near the top of the calyx-tube. Ovary free, sessile 1-2-celled, often truncate at the apex ; ovules 1–α, basal ; style 1 or 2. Capsule membranous below with a hard thick cap which is detached by a circumscissile dehiscence and carries away one or more seeds, 1-2-celled. Seeds 1–α, subreniform ; embryo annular.

Trianthema Portulacastrum Linn. (Specific Description)

A prostrate somewhat succulent herb ; stem more or less angular, glabrous or pubiscent, much branched. Leaves sub-fleshy, Obliquely opposite unequal, the upper one of the pair larger ¾–1¼" by ¾–1¼" the lower ⅜–½ by ¼–¾" broadly obovate, rounded and often apiculate at the apex, cuneate at the base, glabrous ; petioles ¼–½" long much dilated and membranous at the base, especially those of the smaller leaves in which the membranous enlargement forms triangular pouch. Flowers solitary, sessile, almost concealed by the pouch of the petiole. Calyx lobes ovate, acute white, stamens 10–20. Ovary obliquely truncate ; style 1. Capsule small, almost concealed in the petiolar pouch, lid truncate, slightly concealed, with 2-spreading teeth, carrying away at least 1 seed, the lower part 2-5 seeded. Seeds reniform, muriculate, dark black (Fig. 2).

There is another, rather rare, red variety of *Trianthema Portulacastrum* which is sold in the market as Rakta Punarnaba. It differs from the species in having a reddish texture of the whole plant throughout with red margins of the leaves. It is difficult to say at this stage whether Rakta Punarnaba is *Boerhaavia repens* or the *Trianthema Portulacastrum* var. *rubra*. The result of the investigation of these plants will be published later on. Rakta Punarnaba is however very rarely used in medicine.

COMPARATIVE DESCRIPTION

Boerhaavia repens Linn.
(*B. diffusa* L.)

Trianthema Portulacastrum Linn.
(*T. monogyna* L.)

(1) A diffuse non-succulent herb, branches extending laterally to a considerable length from a common-stock, sometimes runs upto several yards, often found growing upon old and broken walls with long branches hanging downwards.

(1) A rather small prostrate or semi-erect somewhat succulent herb, generally not growing beyond 2 ft.

(2) Colour–reddish green

(2) Colour–light green

(3) Root–large, fusiform

(3) Root–small, not fusiform

(4) Stem—prostrate or ascending or descending 2–3 ft., long divaricately branched, slender, cylindric, thickened at the nodes, often purplish.

(4) Stem–more or less angular glabrous or pubescent, much branched $\frac{1}{2}$–1 ft. long.

(5) Leaves in each node in unequal pairs the larger 1–1$\frac{1}{2}$″, the smaller $\frac{1}{2}$–$\frac{3}{4}$″ long both nearly as broad as long, broadly ovate or suborbicular, rounded at the apex, green and glabrous above, usually white minute scales beneath the margin, entire, often coloured pink, somewhat undulate base rounded or subcordate.

(5) Leaves sub-fleshy oblique opposite, unequal, the upper one of the pair the larger $\frac{3}{4}$–1$\frac{1}{2}$″ by $\frac{3}{4}$–1$\frac{1}{4}$″, the lower $\frac{3}{8}$–$\frac{1}{2}$″ by $\frac{1}{4}$–$\frac{3}{4}$″, broadly obovate, rounded and often apiculate at the apex, cuneate at the base, glabrous.

(6) Petioles nearly as long as the blade, slender.

(6) Petiole, $\frac{1}{4}$–$\frac{1}{2}$″ long, much dilated and membranous at the base, especially those of the smaller leaves in which the membranous enlargement forms a triangular pouch.

(7) Flowers very small, shortly-stalked or nearly sessile, 4–10 together in small umbells arranged in slender long-stalked

(7) Flowers solitary axillary, sessile, almost concealed by the pouch of the petiole, colour white.

Boerhaavia repens Linn.
(*B. diffusa* L.)

Trianthema Portulacastrum Linn.
(*T. monogyna* L.)

(8) Bracteoles small lanceolate, acute.

(8) Bracteoles absent.

(9) Perianth (calyx and corolla indistinguishable) $\frac{1}{3}''$ long with 5 lobed margin, lobes plicate, ovarial part of the perianth tube 1/20″ long, contracted above the ovary, glandular viscid ; limb, funnel-shaped, dark pink, with fine narrow vertical bands outside.

(9) Calyx tube (petals absent) with 5 lobes, lobes ovate acute, white.

(10) Stamens 2 or 3, slightly exerted.

(10) Stamens 10–20.

(11) Fruit indehicent $\frac{1}{4}''$ long, clavate, 5–ribbed, viscidly glandular, one-seeded nut, seeds angled.

(11) Fruit smooth circumscessile, dehiscent small almost concealed in the pouch of the petiole, lid truncate non-glandular slightly concave, with spreading teeth, carrying away at least one seed, the lower part 3–5—seeded, seeds reniform, muriculate dull black.

Note.—The red variety is rather a stouter plant with reddish texture of the stem and with red leaves and flowers.

Habitat.—The distribution of both *Boerhaavia* and *Trianthema* is identical over India, Burma and Ceylon.

Summary and Conclusion

Three different types of plants, *viz.*, *Boerhaavia repens*, *Trianthema Portulacastrum* (*T. monogyna*) red and white varieties, are believed to be the Punarnaba of Ayurvedic practitioners. But as a matter of fact the Swet Punarnaba, which is more widely used all over India and which is considered more efficacious, identifies itself with *Trianthema Portulacastrum*, white variety. The white Punarnaba justifies its identity more clearly (Chopra, *Ind. Drug. Ind.*, p. 300) by the presence of white flowers, apart from other parts which are also whitish. In these species only *Trianthema Portulacastrum* (white variety) bears the white flowers. This species is evidently, therefore, the Swet Punarnaba of Ayurveda. The description of Swet Punarnaba as diffused annuals which are grown in rainy seasons and bear white and round leaves and seeds like those of amarantus coincides with the description of *Trianthema* (white variety) and recedes away from the characters of *Boerhaavia repens*. *Boerhaavia repens*, on the other hand, is

a perennial plant and it rejuvenates by its underground tap-root. It is rather a stiff red-stemmed spreading plant which finds a very suitable abode on broken walls hanging sometimes several yards downwards, or when grown over plane surface spreads all around. The characters of *Boerhaavia repens* closely tally with the Ayurvedic description of *Rakta Punarnaba* (Kaviraj B. Kavyatirtha, p. 431–36), as it possesses red flowers, red stem and perennial root stock.

On chemical analysis of the three types of specimens supplied by the writer, Chopra, Chatterjee and Ghosh (1940) have found that the alkaloidal principle ' Punarnavine ' is present in all of them in variable proportions. The percentage of this alkaloid is more in proportion in the red variety of *Trianthema* than either in *Boerhaavia* or in the white variety of *Trianthema* ; the percentage of the salt potassium nitrate is also found to be more in proportion in *Trianthema*, red variety, than in *Boerhaavia* or *Trianthema* white variety. As potassium nitrate is supposed to be partly responsible for the discharge of aquatic fluid from the body of a patient suffering from beri-beri, ascites, dropsy and the like diseases, it will be worth investigation if the red variety of *Trianthema* is more efficacious in the diseases mentioned above.

In conclusion I beg to express my thanks to Bt.-Col. R. N. Chopra, Director, School of Tropical Medicine, for the kind interest he has taken in this work.

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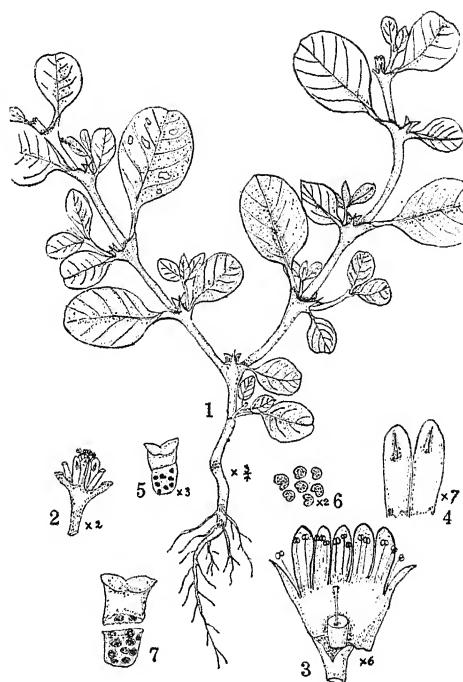
Fig. I.   *Trianthema Portulacastrum* Linn.

1. A flowering plant. 2. A flowering node with a flower. 3. A flower open out to show different parts. 4. Two petals with appendages on the dorsal surface. 5. A ripe fruit showing membranous lower portion and hard solid upper portion with truncated apex. 6. Seeds. 7. A transversely ruptured fruit showing two seeds attached to the upper part and a few in the lower.

Fig. II.   *Boerhaavia repens* Linn.

a. A flowering branch × 1.   *b.* A flower × 5.   *c.* A fruit × 2.

H. L. CHAKRAVARTY—*THE IDENTITY OF PUNARNABA*

THE ANATOMY OF TWO INDIAN FIBRE PLANTS, *CANNABIS* AND *CORCHORUS* WITH SPECIAL REFERENCE TO FIBRE DISTRIBUTION AND DEVELOPMENT

By Balai Chand Kundu, Ph.D., F.L.S.

CONTENTS

Introduction

Studies of fibres of technological importance have relatively rarely been carried out from the botanical standpoint so as to include detailed examination of their distribution, structure and development. Apart from the extremely thorough investigation of *Linum* by Tammes (1907), carried out as a preliminary to genetic studies on this plant, a certain number of workers have published papers, restricted usually to certain features of development or structure, these papers are referred to subsequently as they become relevant to the discussion, after the present notes upon two Indian fibre plants, *Cannabis* and *Corchorus,* are placed upon record.

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In connection with the botanical study, a more intimate study of the cell wall by various physical methods was carried out in collaboration with Dr. R. D. Preston (Kundu and Preston, 1940).

MATERIAL AND METHODS

For the developmental studies plants were grown in Leeds under glass and in the open, and we have also to thank the Regius Keeper of the Royal Botanic Garden, Edinburgh, for his kindness in growing plants of various species from seeds from India. Plants of *Corchorus olitorius* L. were also received from the Director of the Royal Botanic Gardens, Kew. This English grown material was of particular value for the developmental studies, in which growing points and young internodes were fixed in formalin alcohol and other fixatives for subsequent embedding and microtoming, but observations on adult plants were also checked on well-grown material from India, sent to England after preservation. Various methods were employed to macerate tissues for the study of whole fibres but maceration in chromic acid (5% and weaker solutions), or retting in water, were found to give the most satisfactory results. For many stages of wall development, fresh material alone can be used and in this case sections were cut by hand and mounted immediately in the various reagents.

MORPHOLOGY

Cannabis sativa L. is an erect, branched plant normally 4 to 5 feet in height and with a well-developed tap root system. Above the epigeous cotyledons, the leaves are arranged in a decussate phyllotaxis; up the plant the size and complexity of the leaves and the internodal lengths increase until the longest internode, about 18 cm. long is reached about halfway up the stem, this has associated with it the largest leaves, which are stipulate and palmately compound with six or seven leaflets. Above this level the size of the parts falls off again towards the inflorescence. The plants are diœcious, bearing inflorescences in the axils of the upper leaves. The two types of plant cannot be distinguished before flowering, but the staminate are ultimately smaller and die after shedding the pollen, whilst the carpellate plants persist until the fruits are mature.

Corchorus includes the two species *C. olitorius* L. and *C. capsularis* L., which are very similar structurally and mainly distinguished by the form of the capsules (Fig. 1). Though both species supply the jute of commerce, *C. capsularis* is generally regarded as superior and wherever possible is grown in preference to *C. olitorius*, though Watt (1908) remarks that some forms of *C. olitorius* yield as much fibre and fetch as high prices as *C. capsularis*.

C. olitorius is an annual herb, with a relatively long growing season, maturing in September or October. It is indigenous in many parts of India and, though found mainly in Bengal, it has become acclimatised in many parts of India and Burma and occurs as a weed of cultivation. In India it is found on high and dry land.

C. capsularis requires inundation and also has a shorter period of growth, maturing in July.
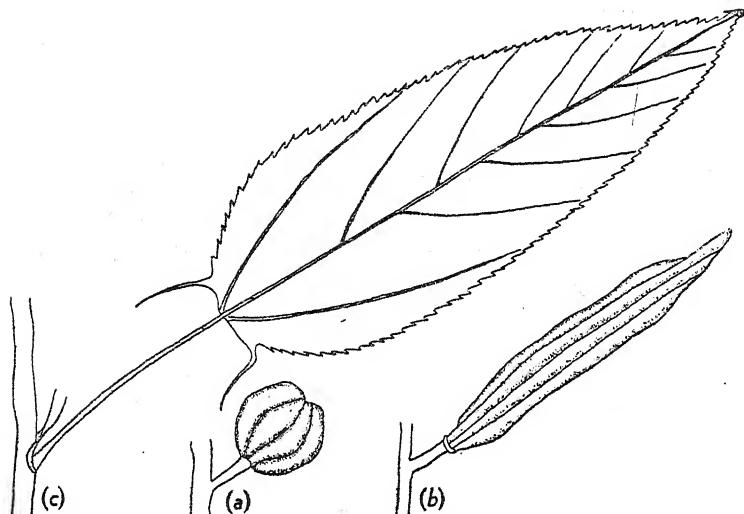
Fig. 1.   Capsules of (*a*) *Corchorus capsularis;* (*b*) *C. olitorius;* (*c*) leaf of *C. olitorius* (× 2/3).

For the present investigation on *Corchorus*, the material has been mainly of *C. olitorius*.   Under normal conditions the plants are 6 to 10 feet in height, though the specimens received from India were between 4 and 6 feet and those from Kew only 3 feet. It may attain a height of 14 to 16 feet when cultivated carefully with suitable manures.   The Indian plants were only branched in the inflorescence region.   The leaves are arranged in a spiral phyllotaxis, which probably varies from 2/5 to $\frac{3}{8}$ according to the vigour of the individual plants.   Each leaf is simple and stipulate and two characteristic attenuated outgrowths are developed from the base of the lamina (Fig. 1).   A considerable number of leaves develop on the plant and the internodes are short, averaging 3 to 8 cm.; there is no uniform increase in internodal length to a maximum as in *Cannabis*, longer and shorter internodes occurring somewhat irregularly.

General Anatomy and Development

Cannabis

The general distribution of the tissues may be considered in a transverse section of the first internode that has just completed extension.   The two most important ribs are present over the median trace bundles of the pair of leaves inserted at the node immediately above, at right angles to these are two broader flanks with three smaller ribs, which overlie the lateral bundles of

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the 3-bundle traces from the pair of leaves above and the median strands of the leaves of the next higher pair. The six bundles from the pair of leaves immediately above may be recognised by the greater proportion of protoxylem and their greater isolation by ray tissue from the synthetic bundles composed of united trace bundles from still higher leaves. In the cambial region the bundles are united into a continuous ring (Fig. 2).
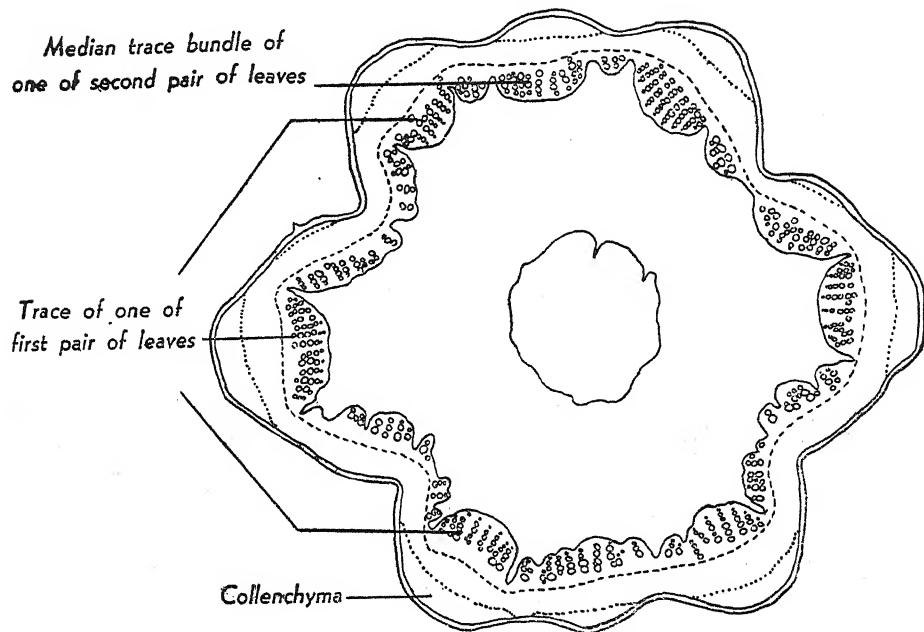
Fig. 2. *Cannabis.* Transverse section of the first adult internode (× 30).

Beneath the epidermis the cortex consists of one to two layers of chlorenchyma with thickened tangential walls, followed by well-developed collenchyma in the ridges and chlorenchyma in the grooves; it is bounded internally by the starch sheath. Immediately within the starch sheath the outermost groups of fibres are seen associated with the bundles; at this stage the fibres are thin-walled, angular in outline and have protoplasmic contents and conspicuous nuclei. The phloem contains a few large mucilage cells and these undergo extreme development in the region of the inflorescence. The pith is large and may become hollow.

The basal regions of older plants become very woody and with the continued radial growth the ridges tend to become less conspicuous. The phloem consists of pyramidal wedges which taper outwards; each wedge consists of about eight to eleven patches of fibres alternating with groups of thin-walled phloem (Fig. 3). The outermost and earliest formed groups are separated from the

fibre-containing phloem formed later by a few layers of paren-
chyma cells.
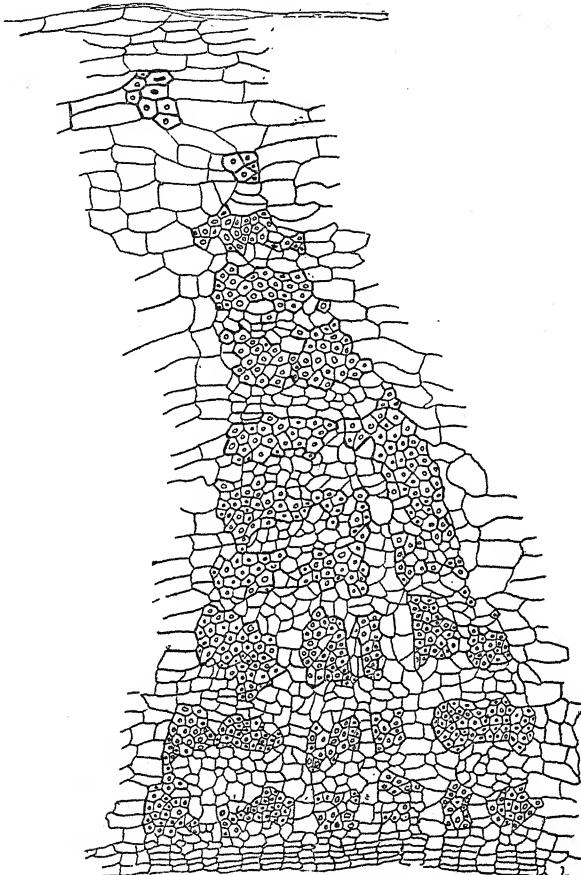
Fig. 3. *Cannabis.* Transverse section of the fibre region of an old
internode (× 130).

A phellogen arises immediately outside the collenchyma in the
ridges and in the sub-epidermal layer in the grooves.

The cells which give rise to the outermost fibres are determined
close behind the shoot apex and the tracing of their origin necessi-
tates an examination of development at the shoot apex. This has
been followed in serial transverse sections 10 μ in thickness.

In the axis below the fourth pair of primordia the prodesmogen
is already interrupted by primary rays to form a ring of strands. It
is in the strands connected with this pair that the first protophloem
was recognised in the axis ; in sections of fresh material the walls of
these elements appear swollen and highly refractive the *nacrée* stage

of Léger (1897) and the contents are seen to be degenerating, whilst in stained sections the walls show marked affinity for fast green. The first phloem elements are differentiated within two or three cells (Fig. 4), sometimes even next to, the layer of larger and more vacuolated cells of the future starch sheath. The first appearance of protophloem elements is followed by differentiation of others in a centripetal direction, whilst the surrounding cells of the prodesmogen strand remain in a relatively meristematic condition. Protophloem differentiation proceeds in this way down to the level of insertion of the seventh pair of primordia ; these phloem elements have been derived entirely from cells of the prodesmogen strand, which are elongated meristematic cells which divide most frequently by transverse walls, but occasionally also by longitudinal walls in any plane, so that the cells assume no characteristic seriation in transverse sections ; in this paper elements differentiated from prodesmogen cells are described as *primary*, so that, down to the level of insertion of the seventh pair or primordia, the protophloem differentiated is primary. The phloem added subsequently is *secondary* since it is differentiated from cells in radial alignment, which have been cut off from the cambium by the characteristic longitudinal tangential divisions.
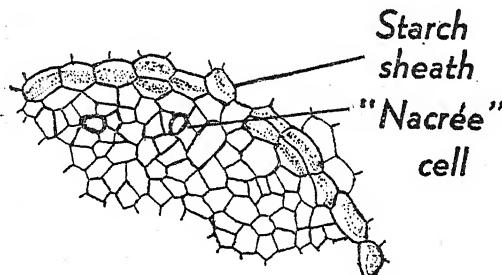
Fig. 4. *Cannabis.* Transverse section of part of young internode showing the position of the first " nacrée " cells in relation to the starch sheath (× 420).

CORCHORUS

In *Corchorus* the leaves are inserted singly and each insertion on a young stem occupies more than half the periphery (Fig. 5). The three most conspicuous ridges, in the internode which is growing in extension, overlie the three bundles of the trace of the leaf immediately above. As the leaves follow one another in a right-handed spiral up the stem, the anodic margin (that towards which the spiral is rising) of the older leaf overlaps the cathodic margin of the next younger leaf, so the other two ridges overlie the median and the anodic lateral bundles of the trace of the leaf inserted at the second node above the level of sectioning. By the first adult internode the bundles are all linked into a continuous ring in the cambial region. The cortex consists of a sub-epidermal layer of chlorenchyma, within which will be found on any radius,

three to six cells of collenchyma and four to seven cells of paren-
chyma. The three innermost layers of parenchyma contain starch
so that the innermost is not clearly defined as the starch sheath.
Conspicuous mucilage cells occur in the cortical and pith parenchyma.
Immediately within the starch sheath occur the fibres, arranged in
groups of twelve to twenty-five and extending over a radial depth
of five to seven cells ; the groups are separated from one another
by parenchymatous rays of one or more cells in width. In this first
adult internode the fibres are angular in outline and most of them
have protoplasmic contents. To the inner side the outermost fibre
groups abut directly upon later developed prosenchymatous
phloem without any intervening parenchyma such as is present in
Cannabis.
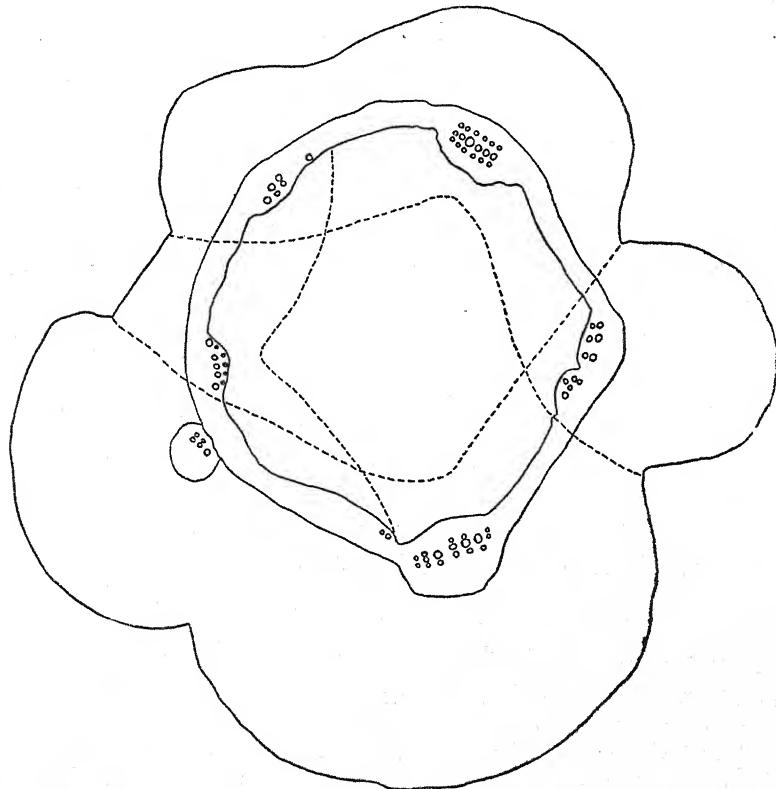
Fig. 5. *Corchorus olitorius.* Transverse section of a young internode.
The dotted lines indicate the regions associated with successive leaf-trace
systems (× 75).

In older stems a woody ring is developed and the pith may
become hollow. The phloem consists of tapering wedges and, as in
Cannabis, the wedges consist of groups of fibres alternating with
typical phloem. No phellogen activity occurs.

Examination of the shoot apex of a vigorous plant of *Corchorus*, by means of serial transverse sections, showed the first differentiation of protophloem and protoxylem in the third youngest primordium. A striking feature of development in this plant is the very early appearance of cambial activity ; at the level of insertion of the third youngest primordium, the central cells of the axis are beginning to vacuolate, whilst surrounded by a ring of more meristematic cells that would normally be described as a meristem ring (Helm, 1931), or a prodesmogen ring (Grégoire, 1935). But already at this level the ring is interrupted by rays which are generally uniseriate and, both rays and meristem have the unusual feature that the cells already show definite radial alignment. The more compressible meristematic cells show this alignment less clearly as, towards the outside, longitudinal divisions are already appearing in other planes in connection with phloem differentiation. The cells of the so-called prodesmogen ring thus appear to have divided almost entirely by tangential longitudinal walls (Fig. 6) so that from the outset it appears to be a cambial tissue and, according to the usage of the terms in this paper, should be described as *secondary*. This view of its nature, based on the transverse view, is supported even more strikingly by the longitudinal view (Fig. 7). It is in this radially seriated tissue that the first protophloem differentiation takes place so that the earliest protophloem is also secondary, but as usual the mother cell of the sieve tube undergoes a few longitudinal divisions before the actual differentiation of the sieve tube, these divisions make the radial seriation rather more difficult to see, but in the rays it persists right out to the starch-sheath (Fig. 6 *b*).
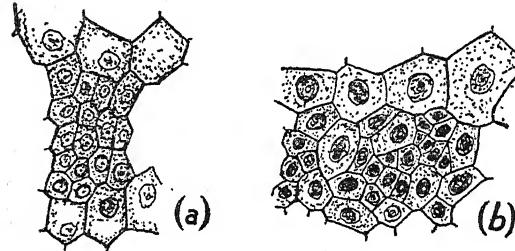
Fig. 6. *Corchorus olitorius*. Transverse section of part of young internode showing evidence of radial alignment of cells of the meristem ring. (*a*) is an earlier stage than (*b*) (× 420).

FIBRE DEVELOPMENT

Origin and early development during extension of the internode.

In *Cannabis* the prodesmogen tissue and in *Corchorus* the first cells derived from the cambium, lie in contact with the vacuolating cells of the starch sheath as seen in Fig. 4. In the meristematic cells of the strand the first protophloem differentiation takes place close within the starch sheath, sometimes in a cell actually abutting upon it. The early differentiation of sieve tubes affects only a proportion
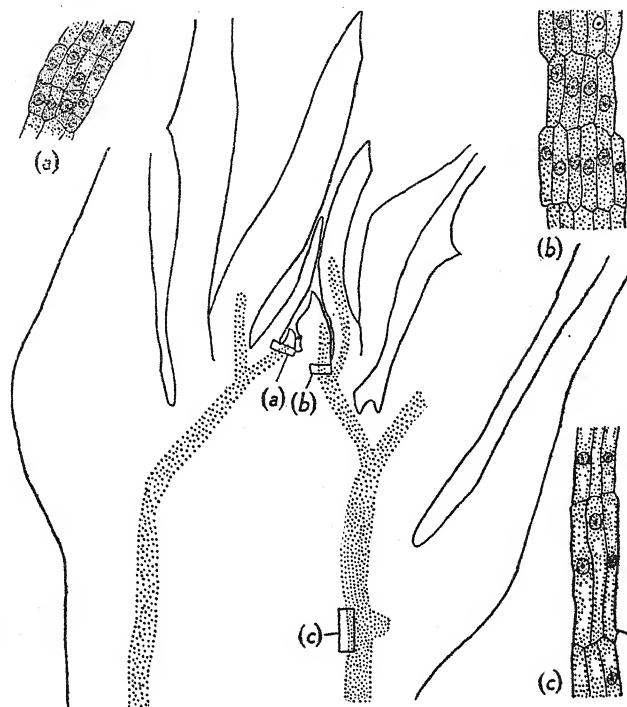
Fig. 7. *Corchorus olitorius.* Longitudinal section of shoot apex (× 55) to show the positions of the meristematic tissues in the inset figures (*a*), (*b*) and (*c*) (× 280).

of the cells of the strand and it is to the remaining cells of the strand that the origin of the first-formed and outermost fibres may be traced. In *Cannabis* below the seventh pair of primordia the future fibres begin to vacuolate and become more rounded in section, with the consequent development of small intercellular spaces between them. At this stage the first-formed and outer sieve tubes are much extended and are collapsing whilst the expanding cells around are also tending to crush them, so that they are gradually obliterated; for a time their former presence may be recognised as what appear to be thickened and deeper stained spots between the cells, but eventually all trace of them is lost. During this stage, longitudinal sections show that the cells amongst the collapsing sieve tubes are elongating with the growth in length of the internode, and all stages from elongating procambial cells to the typical fibres may be followed, leaving no doubt that fibres originate from the protophloem tissue, as described for a number of plants by Léger (1897).

Soon after they begin to elongate the young fibre cells become slightly collenchymatous in appearance, the walls being slightly more thickened and more refractive where they border upon intercellular spaces; this condition is never conspicuously developed, but

may persist for some time. A similar, rather transient, slightly collenchymatous stage was described by Léger for a number of different plants, so that it probably represents a general phase of development of sclerenchymatous fibres.

This stage however differs from the typical collenchyma of the cortex in the fact that, though cellulose reactions are obtained very easily, pectin reactions are not marked; like the typical collenchyma the walls are evidently swollen and the thickenings show well in sections of fresh material in water, but are much less evident after dehydration.

During the phase of rapid extension the young fibres show increase in girth and remain rounded in section (Fig. 8). They have abundant protoplasmic contents and several nuclei (Pl. VII, Fig. 3). The walls are thin but in macerated material the longitudinal walls have localised thicker regions, which are also more refractive and are probably related to the collenchymatous appearance seen in cross section.
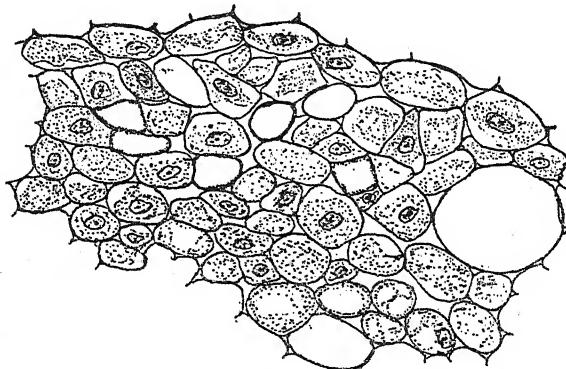
Fig. 8. *Cannabis*. Transverse section showing young fibres developing in rapidly elongating internode. Note the rounded outline and the presence of intercellular spaces. In a few of the fibres the protoplasm is reduced to a thin parietal layer (× 665).

As the internodes complete their extension the outermost fibres cease to grow in length. At the same time the cells undergo further vacuolation, so that the protoplasm is now reduced to a thin parietal layer; the cells increase rapidly in girth and soon reach a cross-sectional area equal to about twice that of the cells during the phase of elongation. The rounded appearance in section is replaced by an angular outline of the cells which now fit closely together without many intercellular spaces (Fig. 9). This appears to be a short phase which is passed through rapidly. The main period of growth in length of the fibres evidently synchronises with the growth in length of the internodes and young fibres macerated from an internode which has just ceased to extend have approximately the same length as the average mature fibres. Growth in girth occurs first at the base of the internode where a section may show the fibres
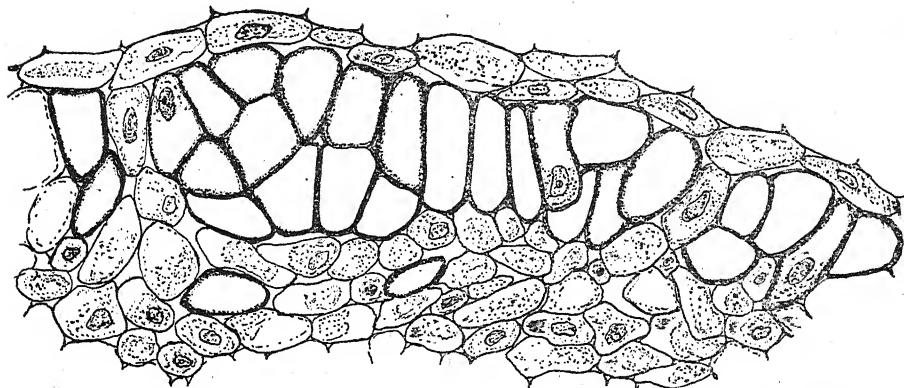
Fig. 9. *Cannabis.* Transverse section showing fibres in young inter-
nodes which has just completed extension (× 665).

expanded and angular, whilst a section from the top of the same
internode may show them still narrow and rounded. At first the
two to four outermost rows of fibres undergo this change but shortly
afterwards the region may increase to a depth of twelve to fourteen
cells and includes the whole of the primary phloem region. A patch
of fibres derived from cells in the primary phloem comprises a
primary fibre group ; these groups are separated from the secondary
fibres by two to four rows of parenchyma cells (Fig. 15).

During all these stages of development the walls of the fibres
are thin and readily give the cellulose reaction with iodine and zinc
chloride; they also swell in 72% sulphuric acid and finally dissolve.
The reaction for pectin with methylene blue, after treatment with
potash followed by washing in water, does not give the characteristic
violet coloration until the last stage, when it is seen well at the
angles of the closely fitting cells and in the middle lamella. No
amyloid stage has been observed in which the wall would give a
blue colour with iodine-potassium iodide alone, but in the collen-
chymatous stage and the next stage the walls give the ' collose '
reaction of a steel-blue colour with iodine after previous treatment
with 15% hydrochloric acid (Ziegenspeck, 1925).

Although most of the primary fibres reach their final length
during the period of extension of the internode, some continue to
increase in length after this time. This is suggested by the fact that
whereas the fibres in more adult internodes have very fine-pointed
ends, a number of the fibres in an internode which has only just
ceased to extend have less finely pointed ends. Evidence supplied
by the pits is also suggestive ; in fully extended fibres the apertures
of the slit-like pits lie in the plane of a very steep spiral, almost
parallel with the length of the fibre, and this is the only type of pit
found in the thickened fibres. In the first extended internode
most of the fibres have this type of pit, but others have pits with
shorter, wider apertures, the long axes of which do not lie along so

steep a spiral. Below the first extended internode, fibres with the wider pits become less frequent and also the pits tend to approach nearer to the long slit type, and finally in the more mature internodes with all the fibres thickened, only the long slit-like type occur (Fig. 10). In extending internodes all the fibres have the type of pit with the wider aperture and lying on a less steeply inclined spiral.
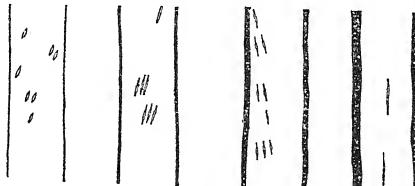
Fig. 10. *Cannabis.* Types of pit on partially thickened, young and adult fibres (× 160).

In *Corchous* the origin of the fibres from protophloem tissue is very clear and since the prodesmogen tissue in this plant is in radial seriation, and therefore cambial in origin from the first, the fibres are all secondary. The initial radial seriation is only seen in places since it becomes lost during divisions of sieve tube mother cells to cut off companion cells and also the cells which give rise to fibres may undergo a preliminary longitudinal division, but the seriation remains very clear in the rays (Pl. VII, Fig. 1). During the early stages of internodal elongation, the cells are densely filled with protoplasm and appear to be in a turgid condition for, on cutting, both the protoplast and the plastic wall collapse. At first the walls appear homogeneous, with slight collenchymatous thickenings at the angles and readily give the " amyloid " reaction. A little later the whole wall appears slightly thicker and the collenchymatous thickenings are no longer visible; this condition is probably due to greater hydration of the inner part of the wall, for it is no longer recognised after dehydration. The inner part of the wall also swells in 5% potash and is thrown into ridges. The walls at this stage give a collose reaction as in *Cannabis.*

In the next stage the walls become less swollen, again during a period of rapid cell extension, and no longer swell appreciably in potash. The cells also lose much of their protoplasmic contents. During the latter phases of internodal extension the collenchymatous thickenings again become visible (Pl. VII, Fig. 2) and persist until the internode has completely finished extension, when most of the protophloem fibres also attain their full length.

Secondary Wall Deposition

The deposition of secondary wall lamellæ starts in the first adult internodes ; in *Cannabis* it is preceded by an increase in protoplasmic contents and some protoplasm persists until the wall thickening is completed ; this is presumably true also of *Corchorus* where wall thickening continues as long as the vegetative shoot remains vigorous.

Once the deposition commences it proceeds rapidly and in *Corchorus* the first lamella is relatively thick and may exceed the thickness of the " primary " wall.   In transverse sections of a fibre group at this stage, fibres with wide lumen, presumably cut near the middle, have thicker walls than those with narrow lumen and the same point is recognised in macerated material (Fig. 11).   This effect is not marked in *Cannabis*, though in macerated material some fibres may be found to illustrate the feature.   In view of Aldaba's work on ramie fibres (Aldaba, 1927), where it was found that secondary lamellæ were laid down first at one end and then progressively along the fibre, in such fibres of *Cannabis* the lamellæ were carefully traced from the region of the thicker wall (Fig. 12), but no evidence was obtained that any lamellæ failed to reach the end of the fibre.   In this case the greater thickness of the wall in the middle regions of certain fibres is probably due to the more rapid

11   12
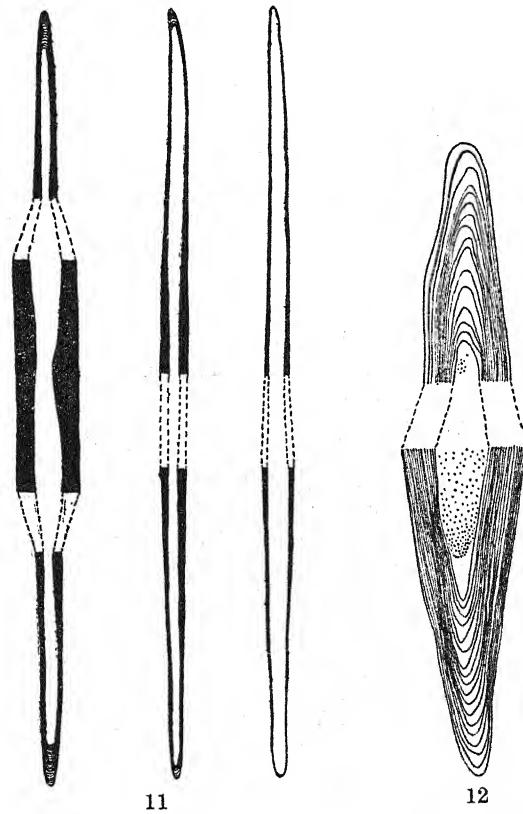
Fig. 11.   *Corchorus*. Fibres showing the wall thicker in the middle region.

Fig. 12.   *Cannabis*. Fibre showing the separation of lamellæ at the ends.

deposition of cellulose resulting in thicker lamellæ, and not to an earlier deposit of additional layers in the middle region of the fibres, particularly as frequently the protoplasmic contents appeared denser in the middle regions of the fibres. Fibres with the wall thinner at one end were never obtained from more adult internodes.

In *Cannabis* the deposit of the first secondary lamellæ can be seen at the proper stage, under high magnifications after careful staining in iron-alum hæmatoxylin, but at first it is evidently much hydrated and is seen most easily in sections of fresh or preserved material, stained in iodine or safranin, without any further dehydration.

At a certain stage in relatively young adult internodes sections of such material show the inner cellulose layers considerably infolded and wrinkled and apparently too large in surface to fit against the outer wall layers. Macerated fibres from the same internode also show unusual appearances which are certainly due to a similar withdrawal and folding of the inner plastic cellulose layer from the outer wall. The reason for these appearances is evidently that the outer wall has been much stretched by extension of the cell under pressure of the vacuolating contents and is therefore under tension; on cutting the stem or on death of the cell, this tension is released and the outer wall contracts. The inner lamellæ, however, deposited since extension and much hydrated, are not under tension and when the outer wall contracts these are thrown into folds. The various patterns of infolds and outfolds may be puzzling to interpret when first seen in macerated material; viewed through the thin outer wall, longitudinal outfolds on the side towards the observer may appear as ridges, infolds as long cracks; the folds may converge or separate, and with the longitudinal folds may be coupled some degree of transverse folding.

In *Corchorus* the more plastic and hydrated condition of the latest deposited lamella is more striking owing to early lignification; lignification starts in the middle lamella and outermost cellulose lamella and each successive lamella also lignifies after it is deposited, so that if a section is allowed to dry at room temperature the innermost lamella contracts away from the older lignified one. Owing to this early lignification, in *Corchorus* only the innermost lamella gives a good cellulose reaction, even in young fibres, though in the region of slip planes the cellulose reaction is clear throughout the thickness of the wall, as apparently the masking lignin material has been displaced from the cellulose surface, as noted by Robinson (1919) in slip planes in wood fibres.

In *Cannabis* secondary wall deposition proceeds at first more slowly in the outer fibres and the wall only appears appreciably thickened in the third or fourth adult internodes; in the later-formed fibres the deposition is more rapid. The thickening continues throughout the life of the plant and is also accompanied by some slight increase in width of the cells, so that the widest and thickest walled fibres are found at the base of the plant, where the cell lumen

may be almost obliterated.   Tammes (1907) also observed in *Linum usitatissimum* that fibres continued to increase in width after wall thickening and during this process became swollen to the rounded form.

In both types the wall shows a lamellated appearance by the fourth adult internode.   Along the fibre the lamellæ are closely superposed and difficult to distinguish, but at the ends they are often more widely spaced (Figs. 11 and 12). This lamellated appearance might be due to one of two causes, either the lamellæ, clearly adpressed along the length of the fibres, are more widely separated at the ends, or the lamellæ, tapering towards the ends where the wall was seen to be thinner at an earlier stage, terminate in succession as a series of cylinders, the inner ones ending progressively further back from the tip as indicated in the diagrammatic Fig. 13. In *Corchorus* the second alternative seems more in accordance with the fact that the lumen of the fibre can be traced far into the end penetrating a large part of the lamellated apex.

The thickening of the wall starts in the outermost fibres and follows in fibres progressively inwards.   In *Corchorus* the thickening of the outermost fibres continues over about the first four adult internodes and then only proceeds very slowly.   In the fourth adult internode the first metaphloem fibres begin to form.
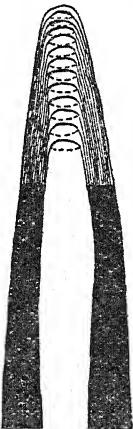
Fig. 13.   Diagram to show lamellæ ending as open cylinders at the end of the fibre.

THE ADULT FIBRES

In both *Cannabis* and *Corchorus* the adult fibres are typical sclerenchymatous cells, long and pointed at both ends.   Those of the outer group have been studied in greater detail and it has been observed in these that, whilst primary fibres of *Cannabis* are multinucleate with 7 to 21 nuclei according to the length of the fibre (Pl. VII, Fig. 3), the secondary fibres and all the fibres of *Corchorus* are always uninucleate.   As the prodesmogen cells of *Cannabis* are

uninucleate, the multiplication of nuclei takes place during fibre differentiation. In young fibres various elongated and constricted nuclei have been seen, which resemble figures given by Saito (1901) for the fibres of *Urtica Thunbergiana*, and described as evidence for amitosis; no mitotic figures have been seen in *Cannabis*. Multinucleate fibres have been described for plants belonging to several different families, including *Linum usitatissimum* (Haberlandt, 1914) but without any suggestions as to the manner in which the condition arose. Esau (1938) has described normal mitosis in fibres of *Nicotiana*.
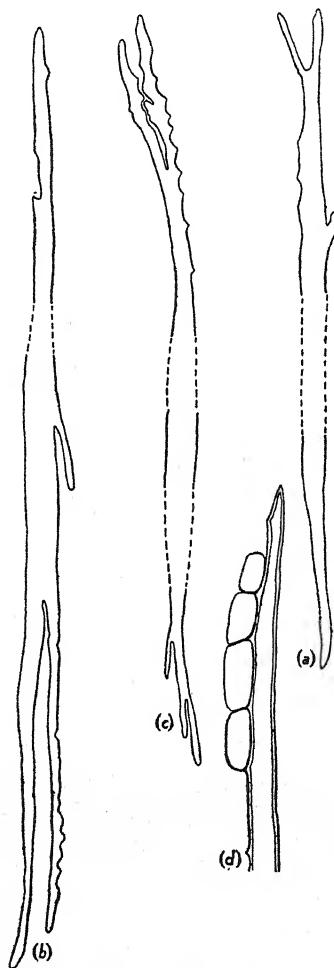
Fig. 14. *Cannabis.* (*a*), (*b*) and (*c*) Peculiar types of fibre (× 70); (*d*) fibre in contact with ray parenchyma (× 210).

The longer fibres in both plants may show a pattern of transverse grooving due to pressure against files of parenchyma cells (Figs. 14 & 17). In *Corchorus* it is not uncommon in older fibres to find an irregular outline to the cell lumen, as though the deposition of secondary lamellæ had not been uniform throughout the fibre and in very old fibres this may lead to actual obliteration of the lumen at certain points (Fig. 17 B & E).

In *Cannabis* the presence of a few layers of parenchyma cells between the primary and secondary fibre groups enables the isolation of the primary fibres by a retting process. It is found that the primary fibres are longer and thicker-walled than the secondary (Fig. 15), and since the primary fibres are growing in length during
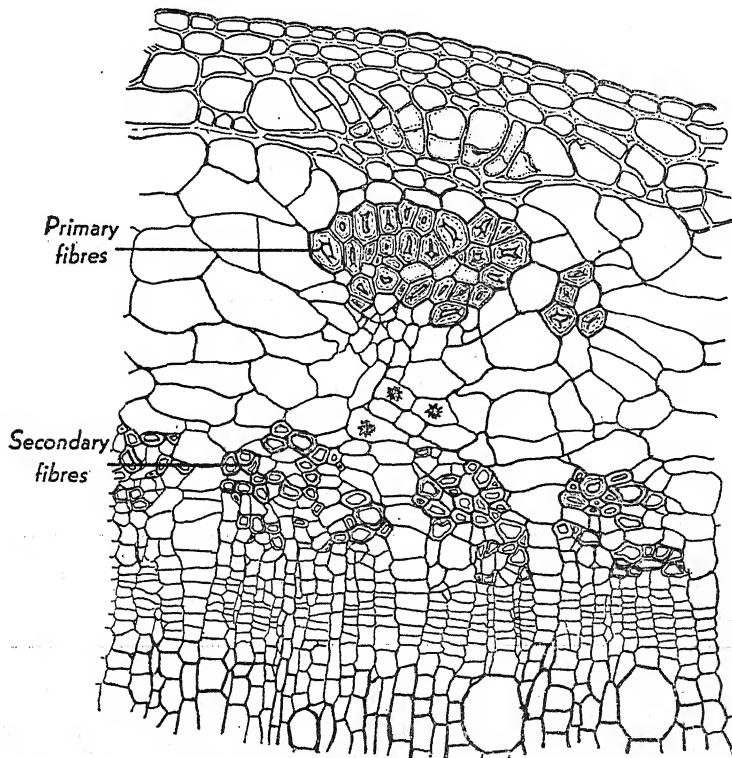
Fig. 15. *Cannabis.* Transverse section of the fibre region of an old internode showing the larger diameter and thicker walls of the primary fibres (× 155).

the period of internodal extension, it is to be anticipated that the longest fibres should occur in the longest internodes. The following figures have been taken from a carpellate plant with nearly mature fruits. The figures given are the average of thirty random measurements :—

		Primary fibres	Secondary fibres
		from near the base of the stem	
Average length	..	12·7 mm.	2·18 mm.
Average width	..	34·2 μ	16·56 μ

The following figures illustrate the variation in dimensions of primary fibres with length of internode. The fibres were taken from a young vegetative plant.

Length of internode	Average length of fibres	Average width
cm.	mm.	μ
2·1	2·77	23·7
5·4	7·36	31·0
8·1	14·1	33·2
6·8	11·8	31·9
10·4	12·4	31·4

In *Corchorus*, all the fibres are secondary and no line of parenchyma separates the protophloem fibres from the metaphloem group. The number of fibres in the peripheral groups derived from protophloem tissues tends to increase upwards to a maximum about halfway up the plant, above which it falls off again. The order of this change may be illustrated from the figures from the small plants from Kew.

Serial Number of internode on the main stem	Number of fibres in outermost groups
19	1750
15	1974
10	2016
5	1361
Lowest internode 1	314

The average length of the fibres of the outer groups (3·156 mm.) is greater than that of the inner ones (1·47 mm.).

As in *Cannabis* the longest fibres occur in the longest internodes but owing to the irregular distribution of intenodes of different lengths in this plant, fibre length does not show any regular increase up the stem.   Fibres between 6 and 4 mm. in length are common in the longer internodes.

The pits are best seen in surface view of fibres from macerated material.   In both plants they are small and slit-like, with the long axis of the slit running almost vertically in the pitch of a very steep spiral.   The pits are frequent along the length of the fibre, but less so towards the ends; in cross-sections corresponding pits are seen relatively frequently between fibre and fibre, but have not been seen between fibre and parenchyma cell.

Cell Wall Reactions

Cell wall reactions have been studied on   sections   cut   from internodes of fresh material and   of   stems   preserved in 70% alcohol and on macerated material.   Since walls of fibres separated by   maceration   with   chromic   acid   lose   some   part   of   their properties, material has been retted under water and primary and secondary fibres carefully separated.   Individual fibre cells from the macerated material   have been teased   out by fine glass needles and used for different reactions.

The following reagents have been employed, aqueous iodine in potassium iodide and 50% sulphuric acid; aqueous iodine and sulphuric acid (3 parts by volume) in distilled water (1 part) and glycerol (2 parts); von Höhnel's reagent (1905); chlor-zinc-iodine (Artschwager's reagent, 1921); phloroglucin and hydrochloric acid; acid aniline chloride; methylene blue (the material being first warmed in 5% potash and then well washed in water).   Swelling reactions have also been studied in various strengths of sulphuric acid and caustic soda.   As a result the following statements seem justified as to the nature of the cell walls.

In *Cannabis* the wall of the fibre consists of cellulose, lignin and pectin.   The lignin occurs as a sheathing material around the cellulose micelles or fibrils and therefore, in untreated material, in the transverse sections of the internodes, the cellulose reaction is masked and the fibres, instead of turning blue, become   dull yellow   to yellowish brown or bluish brown in colour. The lignin reaction is obtained with phloroglucin and hydrochloric acid and on treatment with Eau de Javelle followed by sodium sulphite, according to the method of Cross and Bevan, modified by Norman and Shrikhande (1935), the characteristic pink colour is obtained and subsequently the material no longer colours in phloroglucin and hydrochloric acid.

Lignin occurs much more abundantly in the middle lamella and primary wall than in the secondary lamellæ. The walls of the secondary fibres are more lignified than those of the primary and in these pectin is also found to be abundant in the regions of the middle lamella and primary wall, especially at the corners of the fibres in

transverse section. This is in harmony with the results of Kerr and Bailey (1934) on xylem elements.

On water-retting, the lignin largely disappears from the secondary wall, but sufficient still remains in the primary layer (or outer layers of the secondary wall) to make the cellulose reactions uncertain. Much of the pectin also disappears, particularly from the region in which it is apparently more abundant.

These conclusions are borne out by the swelling behaviour. In transverse sections the outer layers of the wall and the middle lamella show some resistance to swelling in sulphuric acid, probably on account of their high lignin content. This resistance is partially removed on water-retting and completely removed on maceration in 5% chromic acid. After this latter treatment the wall swells uniformly and usually shows no sign of the "ballooning" which may be noticed in water-retted fibres when the outer layers are less swollen in sulphuric acid.

In *Corchorus* the outer and inner fibres give very similar reactions though, on the whole, the inner fibres appear to be slightly more lignified. In all fibres cellulose reactions are masked until the lignin component is removed by appropriate treatment. In all adult fibres also the inner lamellæ of the wall give stronger lignin reactions than the primary wall and the outer lamellæ of the secondary wall. This is the reverse condition to that found in *Cannabis*, and the primary wall also is much less resistant to the action of sulphuric acid or alkali. In caustic soda the walls simply swell, but in sulphuric acid of different strengths they gradually dissolve. In 55% sulphuric acid the fibres swell, the primary wall appearing in the form of spiral fibrils around the secondary. When pressure is applied to the cover glass, the primary wall dissolves and similar spiral striations are visible in the outer layers of the secondary wall. In 60% sulphuric acid the primary wall practically dissolves immediately and, if pressure is applied, the striations in the secondary wall become clearly visible, moving inwards as the wall slowly dissolves except for a thin innermost layer.

In higher concentrations of sulphuric acid the wall practically dissolves immediately except for this thin innermost layer. As a result of the less resistant nature of the primary wall, it is very unusual to get any indication of " ballooning " during swelling in *Corchorus* as the expanding inner layers are not constricted by an outer more resistant layer. After previous treatment with 5% potash, pectin reactions are also given by the fibres, but in sections it is clear that the pectic components are mainly concentrated in the middle lamella.

These micro-chemical reactions in both plants have been preliminary to an intensive study of the method of construction of the cell-wall in terms of modern views on cellulose structure. This work has necessitated extensive observations with the polarising microscope and particularly in connection with the alteration in optical properties of the wall during various swelling processes. This work

is reported in collaboration with Dr. R. D. Preston (1940), it is sufficient for the purpose of this paper to report the general conclusion that the cellulose micellar, or fibrillar, constituent is arranged in the wall in the form of a very steep spiral. Contrary to the usual impression, this seems true of all layers of the wall, the pitch of the spiral being uniform throughout the thickness of the wall; the slits of the pits in the wall have the same spiral pitch.

Fibre Distribution

The section on development has shown that the fibres of *Cannabis* and *Corchorus* in common with those of a number of plants described by Léger, are determined at an early stage of development from cells of the protophloem. Such fibres are frequently described, as for example by Eames and MacDaniels (1925), as pericyclic. Though this would agree with the broad definition of pericycle as the " outermost zone of cells of the stele immediately within the endodermis," it is an unfortunate term, since it masks the very close connection which exists in development between the fibre groups and the leaf trace system of the plant. This connection has in fact not been recognised by Eames and MacDaniels (1925), who state that " the fibrous tissue adjacent to the conducting strand is morphologically not a part of the vascular tissue ".

When cambial activity commences in *Cannabis,* or continues in *Corchorus,* some of the tissues cut off to the outside become typical phloem elements whilst others become secondary fibres. The presence of the groups of fibres in the phloem prevents this tissue from undergoing tangential expansion when the tissues are subjected to strain during radial growth; this strain is taken mainly by the living ray cells which extend tangentially and undergo radial divisions. This widening of the rays between the fibre groups leads to the characteristic appearance of the phloem in outwardly tapering wedges in plants which continue to differentiate fibres in the metaphloem. In tangential longitudinal view the phloem wedges are seen to be linked into a network with elongated meshes, occupied by the rays. When the ray tissue has become much stretched by continued radial growth, some of the fibre groups may be split, thus multiplying the outer fibre groups and throwing the later formed rays into continuity with the cortex. This splitting of the fibre groups also makes them look more diffuse so that their close association with the vascular strands is not easy to recognise, though it is undoubted when followed through the developmental stages.

In *Cannabis* and *Corchorus* the protophloem tissues from which the fibres are derived are continuous from stem to leaf, but the cells amongst the sieve tubes only undergo differentiation into sclerenchymatous fibres where they run in the stem; in the leaf the cells which are potential fibres are recognised as thin-walled prosechymatous cells. Thus if serial sections are followed downwards across a node, it is found that some of the fibres, both of the outermost and of the innermost groups above the node, diverge to either

10

side of the incoming trace bundles and then rejoin, primary to primary and secondary to secondary, to continue their vertical course in the next lower internode. On the other hand fibres developed from the protophloem of the trace bundles themselves, developed in the lower internode, fail to differentiate as the trace bundles pass into the leaf base ; as the fibres begin to die out, they become more widely spaced and tend to occur singly or in small groups (Fig. 16 *a* and *b*).
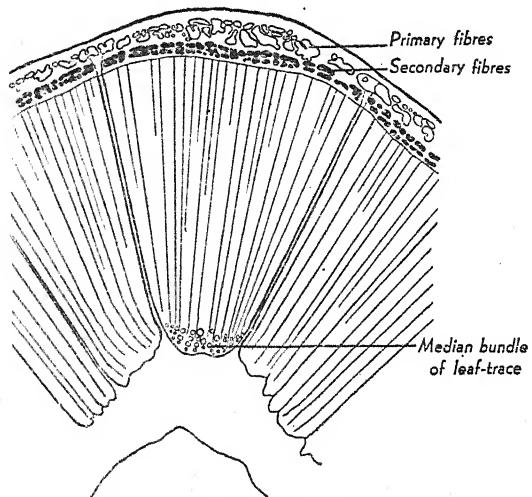
Fig. 16 *a*
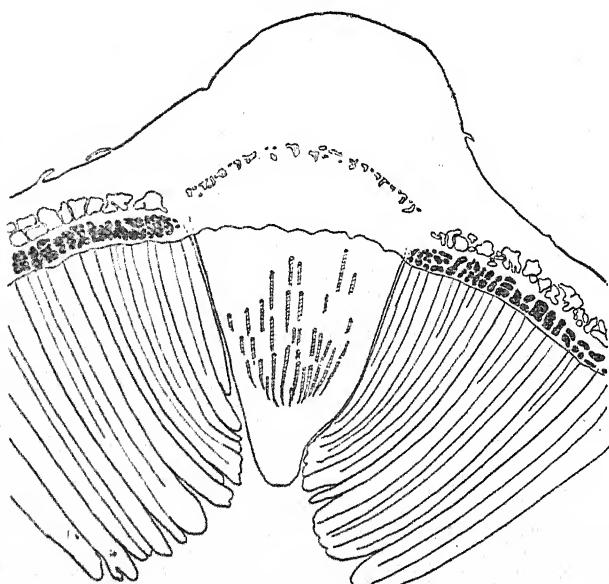
Figs. 16 *b*

Fig. 16. *Cannabis.* Transverse section to show the distribution of the primary and secondary fibres in the region of the median bundle of the leaf-trace : (*a*) in the middle of the internode, (*b*) just below the node (× 15).

The cambial activity in adult internodes, which continues to give rise to additional groups of secondary fibres so long as the plant lives, is limited to the stem. As the trace bundles begin to move out at the node, they gradually emerge from beneath the tissues resulting from this later activity and pass into the leaf with only the xylem and phloem formed during the period of leaf expansion. This is in accordance with the results of Elliott (1933) who found that in the Dicotyledons studied by him cambial activity in the leaf ceased, with completion of leaf expansion. Priestley and Scott (1936), working on *Helianthus*, found this true also of the trace bundles in the internode so long as the bundles remained isolated by the original rays on their flanks. In *Corchorus* and *Cannabis* however, the rays are narrow and the cambium of the bundles of the incoming trace is soon re-activated from the flanks of the vascular ring on either side, though it is noticed that the protoxylem of the bundles is usually separated by a zone of wood devoid of vessels from the later-formed vessel containing wood on their faces.

The total disappearance of fibres from the face of the bundles in the leaf must, however, involve some other explanation in addition, for the peripheral groups of fibres in the stem are formed from protophloem of the trace bundles and this tissue is continuous from leaf to stem, although in the leaf it is represented entirely by soft bast.

As this change is followed through the leaf insertion, it is found that the peripheral fibres first appear where the leaf bundles are about level with the ring of fibres in the stem ; at first the fibres are the most peripheral and are only few in number, but their number is rapidly increased by the addition of more centripetally placed fibres as the bundles pass further into the stem. Thus if the course of the median and lateral bundles is followed as they enter the stem at the node, all these bundles will be seen to bring in with them, on the outer face of the phloem, typical prosenchymatous elements identical with fibre cells in their outline, but without the characteristic thickened wall. In the stem such cells undergo differentiation into fibres, the number of which rapidly increases as the bundles from the leaf enter the stem ; thus in one case in *Corchorus* where four transverse sections, each some $20\,\mu$ in thickness, included the details of the passage of the median bundle of the trace across the phloem of the stem to a position at the outer edge of the xylem ring, the number of fibres on the face of the bundle increased from 36 to 67. This increase in fibres proceeded centripetally, the first to appear were those on the outside of the strand and within these could very soon be found others showing varying stages of wall thickness and lignification, indicating the rapid appearance of further fibres within the original ones. It would appear that in the leaf the phloem elements, which are potential fibres, only differentiate into fibres where they are in close approximation to the fibre-forming tissues in the stem, and it may be that only those elements which are associated with stem fibres at their lower extremities

undergo this differentiation; if this is so it is probable that the development of the most external cells into fibres farthest out into the leaf cushion will be related to their greater length, since being the earlier of the procambial cells to differentiate they will also be those most pulled out by extension.

As the strands take up their position in the ring fibres also develop on their face within the peripheral fibres. These additional fibres of evidently the result of a gradual lateral deflection over the trace, the elements cut off from the cambium of the vascular ring to the sides of the trace strands. Further down the stem such secondary tissue gradually completely encloses the original trace strands, and later-formed fibres, continuous with strands of the internode above, continue to develop centripetally outside the trace. Over the centre of the trace strand near its point of insertion, certain groups of relatively unlignified fibres with only slightly thickened walls may be seen and if these are traced upwards they are found to end blindly and to have no continuity with fibres of the internode above. These fibres seem to differentiate in tissues which owe their origin to the fact that the basipetal renewal of cambial activity in the trace system in the stem also works a slight distance basifugally upwards towards the leaf. This upward renewal of activity ends before the trace bundles enter the petiole and consequently these poorly developed fibres also end at the leaf insertion. In this position a number of cells occur which are transitional between fibres and parenchyma, such as septate fibres, septate prosenchyma cells with only some of the segments thick-walled and fibres with unusually narrow and grooved ends.

Fibres are developed in branch stems, and in longitudinal section it is seen that some of the fibres from the branch are continuous into the main stem and run down the flanks of the main trace bundle of the subtending leaf.

DISCUSSION

The relation of sclerenchyma to pericycle and phloem

Primary sclerenchyma fibres are often described as pericyclic and Morot (1885) even went so far as to conclude that sclerenchyma was derived from special pericyclic layers—a conclusion obviously based upon examination of too advanced stages of development. The fibres lie just within the starch sheath and are therefore in a position to be termed pericyclic, but there also remains no doubt that the fibres arise in the protophloem tissue amongst functional sieve tubes; this fact has been demonstrated by Léger by a developmental study of a wide range of plants and has also been confirmed by Esau (1938) for *Nicotiana* and in the present study of *Cannabis* and *Corchorus*. The use of the term pericyclic is probably responsible for the fact that the very close association of fibres and phloem tends to be overlooked, though further consideration shows that probably the development of fibres is dependent upon the presence of functional phloem in the same tissue. In the case of the outermost

fibres, the associated protophloem sieve tubes are much stretched by tissue extension, collapse early and are obliterated, so that their presence is only confirmed by the study of developmental stages.

This association is not affected by the fact that in *Cannabis* the outermost groups of sclerenchyma are differentiated in protophloem tissue which is primary in origin (derived from prodesmogen tissue), whilst in *Corchorus* the first formed phloem amongst which the fibres form, though undoubtedly protophloem, is differentiated from cells in which tangential longitudinal divisions of a typical cambial type have been proceeding, so that both protophloem and associated fibres are actually *secondary* in origin. It remains true however, that the outermost fibres will differentiate from cells which were growing during the period of stem extension, whilst the inner groups of fibres are derived from cells which are themselves formed after the elongation of the internode has been completed. The processes of growth and differentiation must clearly proceed differently in fibre groups of these two types and indeed there would be considerable convenience in an extension of the prefixes proto- and meta- to sclerenchyma to distinguish those fibre groups formed from protophloem tissue from those formed later from metaphloem.

The relation of sclerenchyma to the leaf-trace system

The old term fibro-vascular bundle is evidence of how closely the fibres have been found to be associated with the vascular strands; the latter are continuous from leaf to stem and usually the whole of the vascular ring in the first year shoot in any Dicotyledon may be interpreted in terms of linked leaf-traces. But in both *Cannabis* and *Corchorus* we have the curious fact that the vascular strands of the leaf, before their entry into the stem, have no fibres on their periphery. Although a common phenomenon, this cannot be generalised too widely, as some Dicotyledon leaves do show fibres in the petiole and even in association with the veins in the lamina. The fibres are always in the same relative position as a cap outside the phloem, and where they are not present, they are replaced by a prosenchymatous tissue, presumably potentially capable of differentiation into fibres. The fact that this potential fibre tissue frequently remains with unthickened walls in lamina and petiole suggests that the carbohydrates synthesised in the adult leaf find their way out of the leaf without being deposited on the walls of the cells amongst, or just exterior to, the phloem of the bundle in which they are probably carried. Why then, after these strands are linked into the trace system in the stem, does cellulose deposition occur and typical fibres appear ?

It has been pointed out by Priestley and Scott (1936) that in *Helianthus*, and the same condition seems general in the Dicotyledons (Elliott, 1933), whilst cambial activity stops in the leaf vascular strand and the isolated strands of the trace as they enter the stem, it continues in the linked system in the axis so long as young leaves, in which cambial growth is active, are associated with the system.

When cambial activity thus occurs in the lower part of the stem, on the flanks of and often on the face of leaf trace systems which ceased activity when the associated leaf became adult, then in *Helianthus*, so long as xylem differentiation continues in the young leaves, additional xylem in continuity with it is also differentiated in the lower part of the stem and the lower internodes increase appreciably in girth. Thus so long as young internodes are adding new wood in the upper regions, roughly equivalent quantities of wood are also added in the lower part of the stem. There is little evidence of continued phloem differentiation on the same scale ; in young internodes phloem is differentiating but there is no suggestion of any comparable increase of phloem in the lower internodes from tissues recently cut off from the cambium. When a leaf high on the stem becomes adult and its phloem is used to transmit its protosynthates downwards into the stem, these supplies may have adequate channels to carry them out of the leaf, but as they reach lower internodes, differentiated in association with lower trace systems, little or no new phloem is being formed here to provide channels for the new supplies flowing in from higher regions. The carbohydrate supplies may find their way into the channels of the older leaf systems, especially as these may not now receive much food from the older leaves, now less vigorous ; but it does not seem surprising that there is considerable leakage or accumulation of carbohydrate from these overloaded phloem channels, and that cellulose is usually deposited in the sclerenchyma on the face of the linked trace systems, although very frequently none is formed in the same vascular strands in their upper regions in the leaf, or where they are still isolated near their entry into the stem.

The deposition of secondary layers in the fibre is a long-continued process. Wall thickening begins to appear in the first adult internode and shows a rapid increase in the next few adult internodes, after which it continues slowly so long as leaf growth continues above and new adult leaves continue to send photosynthates down the stem.

THE STRUCTURE OF THE FIBRE IN RELATION TO DEVELOPMENT

Two phases of growth are distinguished which seem to be associated with different types of wall structure, *viz.*, (1) the phase of cell extension associated with the rapid expansion of an original, " primary " wall ; (2) the phase of cell wall thickening, following upon extension, associated with the deposition of the " secondary " wall.

(1) *The phase of extension of the ' primary ' wall*

It will be necessary in the first place to examine the concept of a " primary " wall. Such an examination may usefully start from the comparatively recent clarification of our terminology of wall layers by Kerr and Bailey (1934). They recognise an isotropic, non-cellulosic, intercellular medium in which the cells are embedded and which forms a layer of middle lamella outside the cellulose proper,

and then distinguish the (cellulose) primary wall as " the cambial wall and its homologues in other tissues ". This wall differs from the secondary wall in its great capacity for growth and extension and ability to undergo reversible changes, *e.g.*, in thickness. The secondary wall in contradistinction, is deposited in cells which have undergone irreversible changes and lost their potentiality for growth and enlargement ; such a secondary wall is deposited in a series of layers and may be conspicuously laminated.

In the fibre cell the two definitions as given by Kerr and Bailey can be applied with great consistency and no difficulty exists in recognising the primary wall stage and following the subsequent deposition thereon of a secondary wall ; at the same time certain considerations suggest that these definitions cannot safely be applied in all tissues. The difficulty probably arises in trying to apply the same terminology of wall layers to walls formed by quite different methods of cell growth, those characteristic of meristem or procambial cells, and of vacuolating cells respectively.

The terms primary wall and secondary wall as used by Kerr and Bailey, apply readily to fibre cells because it seems clear that these cells have a very brief phase, if any, of growth as vacuolating cells. During the stage of rapid cell extension these cells, originally procambial or cambial in origin, retain relatively dense protoplasmic contents. Their very considerable increase in volume is associated with a corresponding increase of surface of the protoplasm which carries on its face an increased cellulose surface, the primary wall. In this extension of the wall there seems to be no question of a " stretching " of the original wall ; no strain is therefore thrown upon the spirally laid cellulose micelles, already present, and no change of pitch results. We can think of the original wall surface as very closely interpenetrated by the protoplasmic surface ; the growth of the cell increases this surface and more cellulose micelles are laid down at the surface, in general conformity of orientation with the micelles already present.

There are several lines of evidence which converge to support this general picture of the process. Wherever this type of wall growth is proceeding, in the apical meristem, the cambium or the young (procambial) fibre cell, the surface of the protoplast is so closely embedded in the cellulose wall that the two cannot be separated as in a vacuolating cell, for instance plasmolysing agents do not bring about the withdrawal of the protoplast and many methods of fixation still leave them closely attached. In the chromic maceration method used in this work the protoplasts are not withdrawn from the wall in the macerated fibres in this phase ; they are always separated in the later stages.

The chemical and physical properties of the fibre walls in this " primary" phase also seem to be in accord with the views thus tentatively advanced as to the nature of the growth process in this primary wall phase. Ziegenspeck (1925 and 1928) described the cellulose wall as passing through a series of phases in development:—

(i) the 'amyloid' phase staining blue with iodine in potassium iodide alone or after previous treatment with Eau de Javelle; (ii) the 'collose' phase, staining steel blue with iodine and potassium iodide after previous treatment with hydrochloric acid, so-called because so frequently given by collenchyma. These two phases are regarded as plastic and when these reactions have been noted in *Cannabis* (only collose reactions) and *Corchorus*, the developing fibres are certainly undergoing rapid extension. Meeuse (1938) has also reported both reactions in the walls of developing Monocotyledon fibres and the existence of a plastic phase during development permits a ready interpretation of certain characteristic features of the adult fibres of *Cannabis* and *Corchorus*. Thus the walls of these fibres have often been impressed by the outline of the turgid vacuolating parenchyma around so that an almost saw-like margin may be produced along one side of a fibre, or a characteristic 'bayonet end' (Fig. 14 *d* and 17).
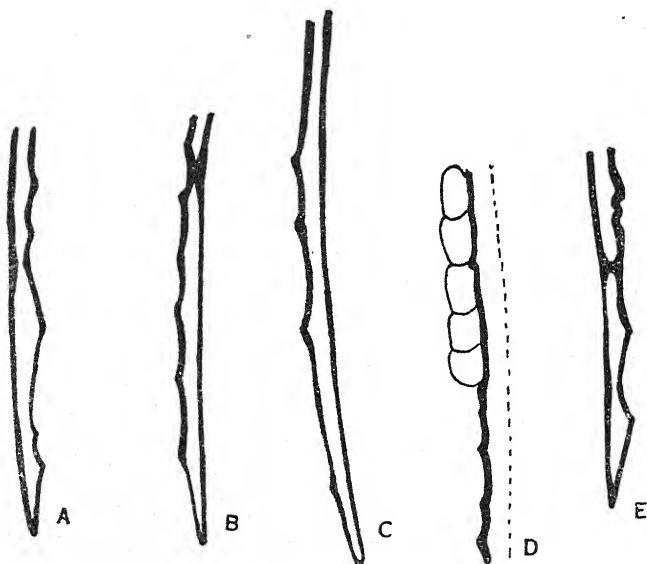
Fig. 17. A.-E. *Corchorus* fibres. "Bayonet" ends and ridges and grooves on fibre walls due mainly to the pressure of surrounding turgid vacuolating parenchyma. In Fig. 17 E some of the parenchyma cells are found attached to the fibre wall in the macerated material.

This impress of the surrounding tissues upon a young fibre would easily lead to the forks and branches so characteristic of *Cannabis* fibres, less common in *Corchorus*. The plastic, young fibre might penetrate the intercellular space between two expanded vacuolating cells and with the continued elongation and division of the parenchyma such a short extension into an intercellular space would be passively drawn out into a well-developed fork or branch.

Another indirect line of evidence that the process of extension of the primary wall takes place at first by a rapid growth process that throws no strain on the original micellar orientation is supplied by the behaviour of the pits.   Throughout the greater part of the period of fibre extension they are relatively broad and lie at a fairly wide angle to the vertical, visible evidence to the unchanging and relatively flat nature of the micellar spiral throughout this stage.

Sliding growth

The picture thus slowly emerging from various lines of evidence, of the extension of a densely protoplasmic element with a soft plastic wall, is entirely inconsistent with such a statement as made by Haberlandt who, speaking of the elongated, awl-shaped ends of bast fibres (1914, *loc. cit.*, p. 153) says " their characteristic form is largely produced by independent apical growth on the part of the individual fibres ; each fibre consequently wedges itself firmly between its neighbours ".

No such mental picture of a thrusting point forcing its way between neighbouring elements is supported by these macerated elements of soft plastic material, which have obviously received a shape impressed upon them and have no rigid surface capable of slipping in the mucilaginous pectin material in which they are embedded. Actually, however, since Haberlandt's statement, founded on the early observations of Krabbe (1886), and based mainly on phenomena of secondary wood development, various critical studies of fibre development have decisively rejected the occurrence of sliding growth during the differentiation of at least the outermost 'protosclerenchyma' fibres.

Tammes (1907) proved conclusively that sliding growth could play no part in the development of the fibres of *Linum*, and Meeuse (1938) has shown recently that no relative displacement of fibres, or even fibre ends, takes place during the extension of various Monocotyledon fibres.   Similarly during this work, it has become clear that at least the greater part of the process of fibre elongation takes place without any relative displacement of the fibres during the process.   As Tammes pointed out originally, if the enormous elongation of these fibres were associated with their movement past one another then the result would be a very rapid increase in their number as seen in cross section ; of this process there is no indication and it can only be concluded once again, that no considerable thrust past one another of the pointed ends has taken place.   In *Corchorus* particularly, a further piece of evidence against such displacement by sliding is provided by the remarkably regular radial seriation of the fibres, even of the outermost groups, the result of their original derivation from cambial activity (Fig. 7).

Quite possibly the different methods of growth of fibre and adjacent parenchyma, one element multiplying by vacuolating cell division, the other extending with a continued increase in its protoplasmic contents, may mean that the adjacent walls of such elements may

move, if slightly, relatively to one another, and this may account for the fact, reported previously, that whilst corresponding pits are frequently found between fibre and fibre, they were not observed between fibre and parenchyma.

The consideration just advanced suggest that the process of fibre extension in later-formed secondary fibres, in ' meta-sclerenchyma ', requires renewed investigation. Here the fibres are formed from cambial derivatives in a region of the stem that has ceased elongation, but the adult fibres are much longer than the cambial cells from which they have been derived.

In *Corchorus olitorius* the lengths of cambium initials have been estimated from measurements of parenchyma files in " strip " preparations [Priestley, Scott and Malins (1933)] and these measurements checked by measurements of the length of vessel segments in macerated material. Wood parenchyma files averaged about 450 μ, vessel segments 465 μ, but the meta-sclerenchyma fibres had an average length of some 1470 μ.

In *Cannabis sativa* the equivalent figures are :—

Cambium initials average length .. 300 μ, width 15 μ

Fibres, average length   .. 2,180 μ, ,,   16 μ.

In both cases, but particularly in *Cannabis*, the fibres appear to have elongated considerably since their original formation from the cambium and the mechanism is by no means clear.   The simplest explanation would be by sliding growth but we are still dealing with densely protoplasmic elements with plastic walls, and slip between these viscous structures is difficult to visualise. Furthermore (in *Corchorus* especially) such slip has been without marked effect on the original radial seriation. In *Cannabis* where the increase in length of cells is sevenfold, the numbers in a group in transverse section should increase sevenfold if elongation is achieved by sliding past one another ; of such a rapid increase in number at this stage of differentiation there is no indication.

In both cases there is the alternative that as the protoplasmic mass of the fibre increases and as the elements deform to the pressure to which they are subjected as they are driven outwards by the expanding tissues of the vascular ring within, the malleable group of cells may deform as a whole, their walls moving as a common framework, a method which Priestley has termed ' symplastic ' (Priestley, 1930) ; in this case there may be considerable displacement of the group as a whole relative to the surrounding parenchyma, but little or no slip amongst the fibre elements themselves. In *Corchorus* the extension of the cells seems to be only threefold and it may well be that the symplastic or sliding adjustment of the walls simply carries originally pointed ends farther in between the tiers of cells immediately above and below. This is somewhat supported by the relatively frequent occurrence, in macerated adult material, of fibres with a median region of wider girth and two more attenuated ends, these three regions being approximately of equal

length. The whole question of the differentiation of these inner metaphloem fibres requires further examination.

It was pointed out earlier that the inclination of the pits in the developing fibres suggested that, towards the end of the process of stem elongation, a phase of growth supervened when fibre extension was associated with a change in the pitch of the cellulose micelles. This stage in fibre growth is probably exceedingly important. In *Cannabis* reasons were given for thinking that this stage was characterised (i) by a relatively rapid increase in width of the fibre (Figs. 8 and 9) ; (ii) by a very intimate mutual association of fibres ; (iii) (when the fibre outline becomes sharply polygonal and intercellular spaces previously visible were practically obliterated) by an increase in fibre volume which appears to be associated with an intake of water rather than with an increase in protoplasmic mass; (iv) and by rapid increase in acuteness of the apex, a feature which may clearly be associated with the mutual compression of the fibres as they expand in width. The increase in sharpness of the apex under these conditions, might well be associated with some degree of mutual slip between the walls, it is therefore very suggestive that in both *Corchorus* and *Cannabis* pits are not usually found very near the apices of the fibres.

Many features thus seen to be associated with this last phase of extension growth suggest that a change is now taking place in the relation of the protoplast to the wall and thus to the type of growth. The protoplast is expanding rapidly and vacuolating, a stage we shall find linked with a capacity of the protoplast to plasmolyse or to shrink away from the wall in fixing or macerating agents. Along with this looser connection of the wall and protoplast, the increase in surface of the cell is no longer associated with a proportionate increase in the protoplasmic substance ; the increase in surface throws the existing wall under strain, the pitch of the micelles and of the pits alters, and now immediately we shall find that additional cellulose deposition means the formation of new lamellæ within the existing wall, and thus begins the deposition of lamellæ of the secondary wall upon the primary wall.

(2) *The phase of deposition of the secondary wall*

Up till now, probably, as Kerr and Bailey suggest, wall thickness has not necessarily remained constant, there have been evidences of ' collenchymatous ' thickening, the wall may have thickened during a slower period of growth and been thinned again during a period of rapid increase of surface. The more fundamental fact is that one method of wall formation, linked with intimate connection between protoplast and wall, is now replaced by another in which a cellulose layer is formed over a free protoplast surface (and not therefore at places where the protoplast is still attached by plasmodesma, so that these places soon become more conspicuous as thin-pitted areas in the thickened wall), between this surface and the pre-existing wall. It would seem inevitable that this mode of wall formation should

give a lamellated structure, often with visible laminations, in which the new layers should be different in many ways from the original primary wall and that the union between the two might not be so intimate. Many of the observations previously recorded are in accordance with these deductions from development.

In *Cannabis* for instance the micro-chemical observations show the primary wall as more lignified and, as a result, giving less definite cellulose reactions on direct treatment with cellulose reagents, and still more striking is the behaviour of water macerated fibres in swelling reagents. The primary wall is then shown to have much less capacity to swell ; it never shows a laminated structure on swelling like the secondary wall and as a result of the greater swelling of the inner secondary layers it is burst by a slit which runs parallel to the micellar direction. The burst layer may remain wound around the swelling inner layers which thus form balloon-like regions between the lines of the spiral of the primary wall. A careful and very precise use of graduated strengths of sulphuric acid enable preparations to be obtained in which this outer primary wall is entirely freed from the inner layers ; it then returns to its original form and micellar pitch. Upto the present no method has succeeded in thus separating the primary wall of a *Corchorus* fibre from the inner walls but there again the two wall layers are undoubtedly different and in young fibres at a certain stage of development the two layers are often separated as a result of the contraction of the stretched primary walls after sectioning or maceration. The recently deposited secondary wall layers are evidently in a very plastic condition and, at this stage of development only, they readily separate from the outer layer and, as they do not contract when this contracts, they are thrown into folds within the fibre lumen. Anderson (1927) has explained this phenomenon as evidence for an extraordinary process of wall deposition. He assumes that successive layers of cellulose are first formed in this loose position over the protoplast and then pressed out flat against the inner surface of the stretched fibre wall, very much as a bill sticker flattens out a poster against a bill-board. There can be no doubt that each layer of cellulose is first formed at the surface of the protoplast and so this surface is assumed contracted away from the wall in a plasmolysing strength of sugar solution when the wall first forms and then as driving the layer against the outer wall when the cell once more becomes turgid. This idea, that the wall should first be formed over a collapsed contracted protoplast and then flung against the outer wall, has no probability and derives its only support from observations of this type which have just been dealt with and which are adequately explained on the lines just indicated. Anderson's explanation would leave a far more important and more widespread phenomenon without explanation, that is the ultimate connection between the micellar patterns of successive lamellæ. This is discussed in a later paragraph.

Quite apart from the observations of contracted inner layers in young fibres, adult fibres from chromic macerated material sometimes show the secondary wall separating from the primary ;

Priestley and Scott (1939) have figured one in the fibre of *Lycopersicum*. It would seem that fairly frequently a readily soluble layer, probably containing an unusually high proportion of pectin, separates the primary wall from the secondary and thus enables these two layers to be sharply differentiated by various treatments.

To return now to the point about the secondary wall deposition which Anderson's theory would leave completely without an explanation ; this is the remarkable consistency in micellar orientation in successive layers, whether primary or secondary, throughout the thickness of the whole wall. This is not generally recognised as a characteristic of the fibre wall, exactly the opposite is stated by Steinbrinck (1927) and repeatedly quoted by other workers, but in these two fibres the result of an intensive examination leaves no doubt on the point and puts in question all the other statements based, as they practically invariably are, upon the employment of swelling techniques for the purpose of observation.

This constancy in the molecular arrangement of cellulose throughout the thickness of the wall in *Corchorus* and *Cannabis* requires explanation. The obvious suggestion would be that, though laid down on the surface of the protoplast, each new layer is formed in contiguity with those previously formed and that the micellar orientation is determined by that in the layers already present. If this conclusion is substantiated, then micellar pitch, probably the most important single factor in fibre construction in reference to its industrial utilisation, will have proved to be determined by growth conditions, because, as we have seen, the original micellar pitch of the primary wall remains unaltered during most of the period of extension and then is modified by the brief growth period during which surface increase is not associated simply with increase of protoplasmic mass.

In *Corchorus* lamellation of the secondary wall, often quite unnoticeable throughout the length of the fibre, may be conspicuously shown in the tip. This may be due to the fact that as the protoplasmic contents of the fibre diminish during the process of deposition, they are withdrawn somewhat from the ends and successive lamellæ here follow one another at a greater distance ; Franz (1934–35) has pointed out how the successive layers in the tips of the hair of *Humulus Lupulus* may thus show very plain lamellation, (even with débris of protoplasm sometimes left between successive layers) or hourglass-like formations may be produced ; Krabbe (1887) described the wide lamellation of the blunt ends of the fibres of *Euphorbia palustris*.

Throughout this paper a well thickened fibre from an adult internode has been referred to as an adult fibre. In *Cannabis* and in the specimens of *Corchorus* from India, there was distinct evidence that the fibres in the basal internodes were wider than any other fibres, and the evidence is good that these fibres retain some protoplasm throughout the life of the plant and continue to deposit internal layers of cellulose and to increase in girth so long as the plant is

vigorous and therefore so long as carbohydrates are being returned to the base from the leafy apex. It is quite unthinkable that the increase in girth of this already much thickened fibre should be due to the swelling energy of its protoplast. But the layers of wall continually deposited may obviously be the seat of development of considerable inter-micellar forces, sufficient to expand the outer layers of the wall.

SUMMARY

Details are given of fibre structure and of the processes of growth and differentiation associated with fibre production in *Cannabis sativa* and *Corchorus olitorius*.

The fibres are shown to develop, in both plants, from elongated cell elements amongst the phloem, protophloem and metaphloem. In *Cannabis* the outermost groups may arise from primary elements; in *Corchorus* all fibres arise from elements of secondary origin.

Fibre development progresses in two stages: (i) extension without new layer deposition—the primary wall phase, (ii) deposition (in layers) of the secondary wall. Deposition of the secondary wall only occurs in the stem after elongation has ceased.

Growth in the primary wall phase is discussed, sliding growth is rejected for the outermost (proto=) fibre groups, but more observations are necessary on the mode of growth of the inner (meta =) fibre groups which elongate in an internode which has itself ceased to elongate. Various features of fibre form, forks, branches, etc., are shown to be connected with the nature of this primary wall growth phase; the change in micellar pitch and in the angle of the slit of the pit is shown to occur in the final cell adjustments when this primary wall growth phase passes into the secondary wall growth phase.

The distinctions between primary and secondary walls are examined and also the various ways in which these walls may be separated, either in developing or adult fibres.

The deposition of secondary layers seems to occur simultaneously over the whole fibre, not to proceed gradually along the fibre in a series of lamellæ as described by Aldaba for *Bœhmeria* and *Linum*.

As the micellar pitch is constant throughout the fibre wall thickness, it appears to be determined by the pitch finally taken up by the primary wall before secondary wall deposition commences. Thus the factors of development governing this change of pitch would appear to determine what is probably the most important physical constant in connection with the industrial utilisation of the fibres.

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DESCRIPTION OF PLATE FIGURES

Fig. 1. *Corchorus olitorius.* Transverse section of a young adult internode, showing radial seriation across the vascular region especially in the rays (× 370).

Fig. 2. *Corchorus olitorius.* The second collenchymatous stage in the fibre development (× 370).

Fig. 3. *Cannabis.* Part of a young fibre from macerated material, Two elongated nuclei are seen in the protoplast (× 400).
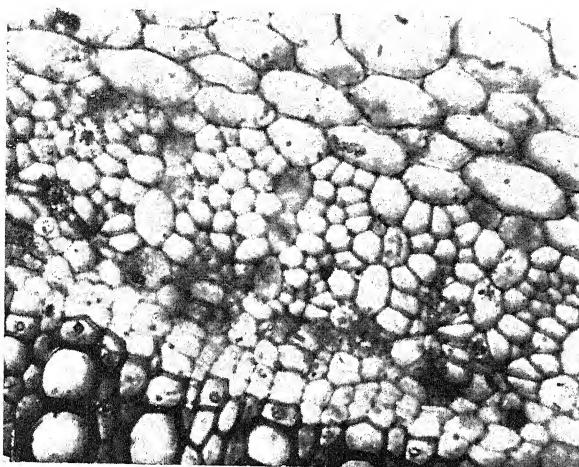
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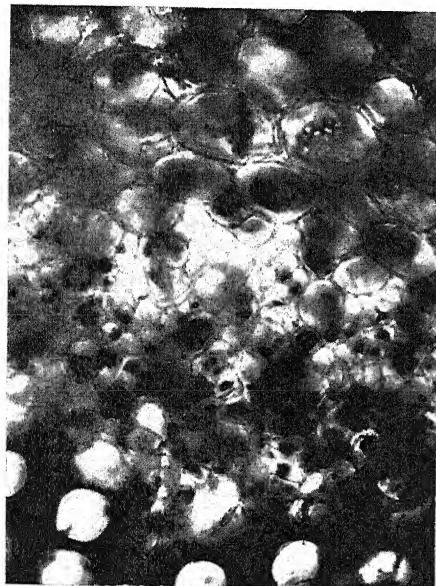
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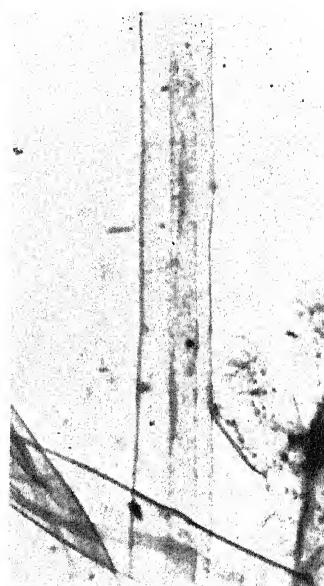
Fig. 3

BALAI CHAND KUNDU—CANNABIS *AND* CORCHORUS
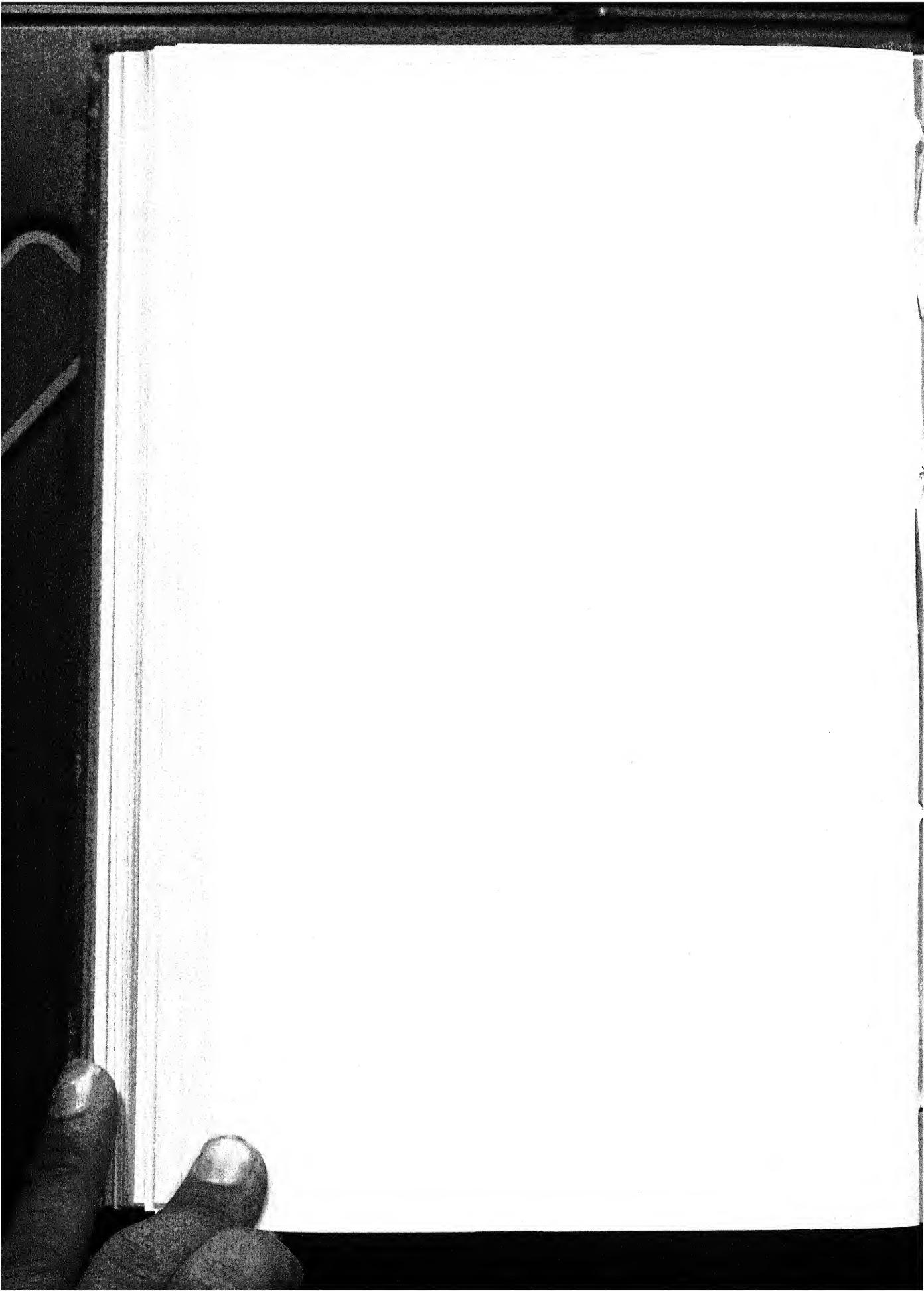
ON THE OOGAMOUS SEXUAL REPRODUC-
TION IN A *CARTERIA**

By K. R. Ramanathan

University Botany Laboratory, Madras

(Communicated by M. O. P. Iyengar)

THE alga forming the subject of this communication was collected in one of the temporary rain-water pools formed on the sandy beach at Madras in December 1940, during the N.E. monsoon. It was found in large numbers on moist sand very near the water edge giving the wet sand a greenish appearance. When the wet greenish sand was collected and shaken with some water, the water became immediately quite green in colour. A drop of this water when examined under the microscope showed numerous individuals of the alga along with *Pandorina, Scenedesmus, Cœlastrum,* etc. The cells of the alga were quite healthy and bright green in colour, but most of them were quiescent and had no cilia, only a few showing active movement. When they were kept for a short time in the water they developed cilia again and began to show active movement. These algæ were found inside the pool also, but their number was comparatively very small. Iyengar (1920, p. 333 ; 1933, p. 324) found on the moist sand fringing the temporary rain-water pools in the Madras beach a number of unicellular and colonial Volvocales, giving the moist sand a greenish appearance. He suggested that these algæ preferred this situation presumably due to the greater aeration and the lower temperature of the wet sand than inside the pools.

VEGETATIVE CELLS

The cells of the alga are ovate to ellipsoid in shape and rounded both at the anterior and the posterior ends (Text-fig. 1). Occasionally the anterior end is somewhat slightly truncate. The cell membrane is thin and smooth and closely enveloping the protoplast. No papilla could be seen. The cilia are as long as, or slightly longer than, the length of the body. The shape of the chromatophore could not be made out clearly owing to the dense nature of the chromatophore and also the accumulation of starch inside, but appeared to be somewhat bell-shaped with a small anterior opening. A large median pyrenoid is embedded at its posterior end. A bright linear eyespot is situated a little from the anterior end (Text-fig. 1). Two contractile vacuoles are seen just below the insertion of the cilia. The nucleus is located just above the median portion inside the hyaline cytoplasm.

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* From the Department of Botany, University of Madras.

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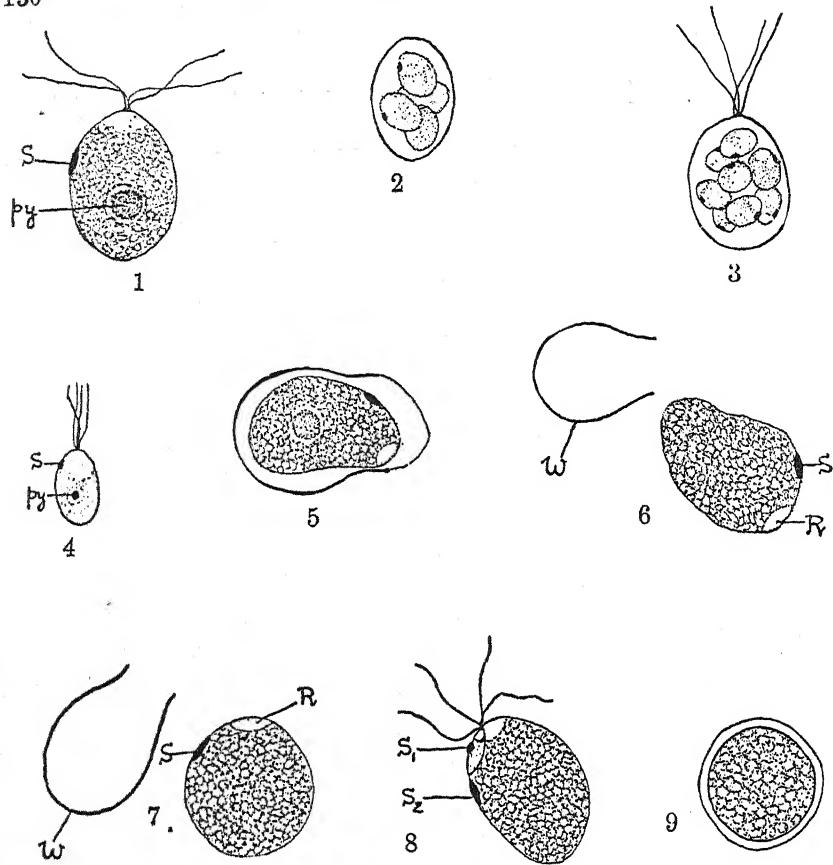
Text-figs. 1-9.—*Carteria Iyengarii* sp. nov. Fig. 1. Vegetative cell. Fig. 2. Four male gametes formed in a cell. Fig. 3. Eight male gametes formed inside a cell which still retains its cilia. Fig. 4. A male gamete. Fig. 5. A female cell showing the gelatinization of the wall at the anterior region and the egg about to escape out. Fig. 6. Egg just escaped out of its mother wall by amœboid movement. Fig. 7. Egg rounded after escaping from the mother wall. Fig. 8. Fusion of the male gamete with the egg: note the two eyespots of the gametes and the four cilia of the male gamete in the zygote. Fig. 9. Zygote surrounded with a wall. (All figures × 950.) (*s*, eyespot; s_1 & s_2, eyespots of the male and the female gamete, respectively; *r*, receptive spot; *w*, discarded wall of female gamete; *py*, pyrenoid.)

REPRODUCTION

Asexual reproduction was not observed in the material. But sexual reproduction takes place through the fusion of a motile quadriciliate antherozoid with a non-motile egg.

Male gametes :

Some of the vegetative cells after coming to rest and losing their cilia form two, four or eight male gametes which finally escape

outside through the gelatinisation of the mother wall (Text-fig. 2). The cells giving rise to the male gametes are somewhat brown in colour in contrast to the bright green colour of the ordinary vegetative cells. These male gametes are pear-shaped or slightly rounded and are much smaller than the smallest vegetative cell (Text-fig. 4). They are four-ciliated and bright golden brown in colour and possess a pale green chloroplast with a small pyrenoid at the posterior end. A bright red eyespot is seen a little from the anterior end (Text-fig. 4). Quite often the male gametes are formed in cells which still retain their cilia (Text-fig. 3).

Female gametes :

In contrast to the male gamete, the female gamete is formed by the transformation of the entire contents of a single vegetative cell into a large non-ciliated egg (Text-figs. 5–7). The cell giving rise to the egg, after coming to rest, loses its cilia and becomes more bright green in colour. It retains its eyespot, contractile vacuoles and cell-membrane.

Fertilization :

The male gametes, soon after their escape from the mother cell, swim towards the female cells and swarm round them in large numbers. During this swarming, the wall of the female cell at the anterior region gelatinizes (Text-fig. 5) and the entire contents escape out of the wall as a large naked egg by a somewhat amœboid movement (Text-fig. 6). After escaping outside it again becomes rounded and in that condition shows clearly the eyespot, the two contractile vacuoles and a somewhat hyaline region at the anterior end representing the receptive spot (Text-fig. 7). One of the motile male gametes finally fuses with the naked egg at the region of the receptive spot (Text-fig. 8). The fusion is very rapid, the entire process not exceeding 10–15 seconds. The fertilized egg shows the two eyespots of the male and the female gametes and slowly moves about for some time with the aid of the four cilia of the male gamete (Text-fig. 8). The place where the male gamete fused with the female gamete could be seen as a small brownish area in contrast to the bright green colour of the rest of the zygote. Finally the cilia are lost and the zygote settles down and forms a wall round itself. The fully formed zygote is spherical and somewhat brown in colour and possesses a thick smooth hyaline wall (Text-fig. 9). The further fate of the zygote is not known.

DISCUSSION

So far in all the previously investigated species of *Carteria*, the sexual reproduction is either typically isogamous or only slightly anisogamous. According to Pascher (1927, p. 139) extreme heterogamy is not known in *Carteria*. The present alga is, therefore, very interesting in showing an extreme form of heterogamy, in fact definite oogamy, a type of reproduction so far recorded in only a few members of the unicellular Chlamydomonadaceæ.

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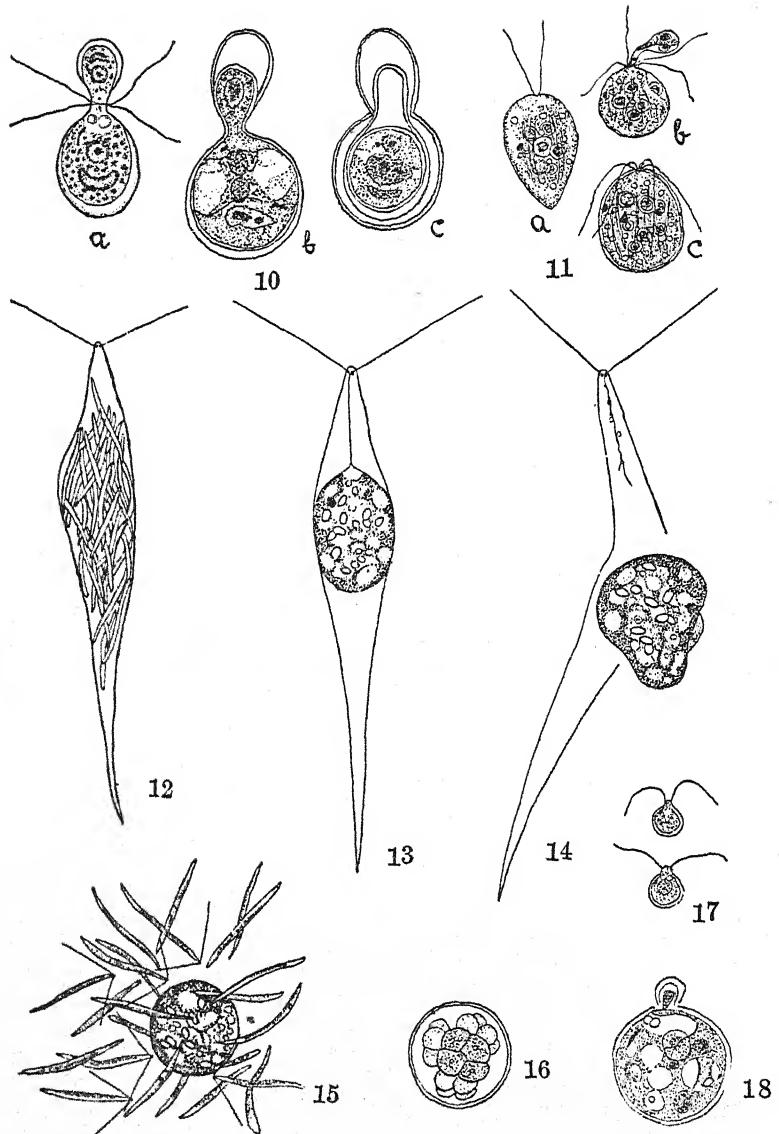
Text-figs. 10–18.—Sexual reproduction of the heterogamous and oogamous unicellular Chlamydomonadaceæ. Fig. 10 (*a–c*). *Chlamydomonas Braunii* Goroschankin. Stages in conjugation of gametes. Fig. 11 (*a–c*). *Phyllariochloris striata* (Korschikoff) Pascher. *a*, vegetative cell; *b*, fusion of two gametes; *c*, zygote. Figs. 12–15. *Chlorogonium oogamum* Pascher. Fig. 12. Format on of male gametes. Fig. 13. Formation of the ovum. Fig. 14. Liberation of the ovum. Fig. 15. Fertilization. Figs. 16–18. *Chlamydomonas coccifera* Goroschankin. Fig. 16. Formation of male gametes. Fig. 17. Male gametes. Fig. 18. Fertilization. (Figs. 10, 11, 16–18, redrawn from Pascher, 1927; Figs. 12–15, redrawn from Pascher, 1931.)

Only about four members of unicellular Chlamydomonadaceæ show extreme heterogamy or definite oogamy, *viz., Chlamydomonas Braunii* Goroschankin, *Chlamydomonas coccifera* Goroschankin, *Phyllariochloris striata* (Korsch.) Pascher (=*Phyllomonas striata* Korsch.) and *Chlorogonium oogamum* Pascher, all the remaining forms being either isogamous or anisogamous.

In *Chlamydomonas Braunii* (Goroschankin, 1890) eight biciliate, walled microgametes are formed from each cell and four very large, biciliate, walled macrogametes from some other cells. During conjugation, the walls of the two gametes coalesce at their anterior region and the contents of the microgamete pass into the macrogamete and fuse with it (Text-fig. 10). The cilia are lost during conjugation. The zygote secretes a new wall in the usual way.

In *Phyllariochloris striata* (Korschikoff, 1925; Pascher and Jahoda, 1928) the sexual reproduction is somewhat similar. 16, 32 or 64 biciliate, naked male gametes are formed in a cell. These are rounded at first but later on become somewhat elongated like a spermatozoid. Two or four large biciliate walled female gametes are formed from other cells. The zygote formed by the fusion of the two gametes, retains the four cilia for a long time (Text-fig. 11).

In *Chlorogonium oogamum* (Pascher, 1931) a large number of biciliate male gametes is formed from a cell (Text-fig. 12). These are narrow and very much elongated like typical spermatozoids. The female gamete is formed by the transformation of the entire contents of a vegetative cell into a single non-ciliated ovum, which escapes out of the mother-cell-wall by an amœboid movement (Text-figs. 13, 14). Fertilization takes place outside the mother wall (Text-fig. 15).

In *Chlamydomonas coccifera* (Goroschankin, 1905) 16 spherical biciliate male gametes are formed in a cell (Text-figs. 16, 17). An ordinary vegetative cell by losing its cilia and becoming considerably enlarged forms the female gamete. Both the male and female gametes are clothed with a cell-wall (Text-fig. 18). During conjugation the male and female gamete come together and the walls of the two gametes become contiguous at their anterior ends (Text-fig. 18). The contents of the male gamete pass into the female cell and fuse with it and a zygote is formed.

Of these four forms, in the first two, *viz.*, in *Chlamydomonas Braunii* and *Phyllariochloris striata*, the micro- and macrogametes are both formed by the division of the vegetative cell and both are ciliated until the time of conjugation. They should be considered therefore as cases of extreme heterogamy, very nearly approaching oogamy. On the other hand, in the two latter cases, *viz., Chlorogonium oogamum* and *Chlamydomonas coccifera*, the female gamete, which is formed by the transformation of the entire contents of a single cell without division, is non-ciliated and constitutes a definite ovum or egg. These two cases should therefore be considered as typically oogamous. Of these two again, in *Chlorogonium oogamum*

the egg escapes out of its mother-cell wall by an amœboid movement and is fertilized outside the parent cell-membrane. In the case of *Chlamydomonas coccifera* the egg does not escape outside and fertilization takes place inside the female cell. The sexual reproduction in this alga should be considered as more advanced than in *Chlorogonium oogamum*, since its egg is completely non-motile and does not show even the slight movement shown by the egg of the latter alga when escaping out of its cell membrane. *Chlamydomonas coccifera* should be considered therefore as showing the highest expression of sexual reproduction among the unicellular Chlamydomonadaceæ.

As regards the present alga, its sexual reproduction is very similar to that of *Chlorogonium oogamum*. Here also the entire contents of a single vegetative cell are transformed without division into a single non-ciliated egg which escapes out of its parent wall by an amœboid movement and is fertilized outside it. However, in the present alga, the male gametes, though small, still retain the shape of the ordinary vegetative cells, in contrast to the typically spermatozoid-like male gametes of *Chlorogonium oogamum*. Therefore, the present alga should be considered as slightly less advanced than *Chlorogonium oogamum* in sexual reproduction.

Regarding the specific position of the present alga, it is quite different from all the other species of *Carteria* recorded before, not only in its morphological features, but also in having oogamous sexual reproduction. It is therefore best to consider it as a new species which may be named as *Carteria Iyengarii* sp. nov.

DESCRIPTION

Carteria Iyengarii sp. nov.

Cells ovate-ellipsoid with a broadly rounded posterior end : anterior end broadly rounded or slightly truncate : membrane thin ; papilla absent ; cilia as long as the length of the body or slightly longer ; chromatophore bell-shaped, almost covering the entire cell, with a single median pyrenoid placed in the posterior region ; eye-spot linear, placed a little distance from the anterior end ; contractile vacuoles two, just below the cilia ; sexual reproduction oogamous, by the fusion of a small four ciliated male gamete with a large non-ciliated egg ; two, four or eight naked male gametes formed inside a cell ; male gametes four ciliated, brown in colour, rounded or ellipsoid in shape with an eyespot and a small chromatophore with a pyrenoid ; female gametes formed singly by the escape of the entire protoplast as a naked egg ; egg non-ciliate, with an eyespot, two contractile vacuoles and a hyaline receptive spot ; zygote rounded with a thick smooth wall. Vegetative cells $20 \cdot 0$–$25 \cdot 0 \, \mu$ long and $15 \cdot 7$–$20 \cdot 0 \, \mu$ broad ; male gametes $10 \cdot 0$–$11 \cdot 7 \, \mu$ long and $6 \cdot 7 \, \mu$ broad ; zygote $20 \cdot 0$–$21 \cdot 70 \, \mu$ in diameter.

Habitat.—On moist sand near the water edge in a temporary rain-water pool on the sea-coast at Madras, South India.

SUMMARY

An account is given of the sexual reproduction in *Carteria Iyengarii* sp. nov. from Madras. The sexual reproduction is of a high type in being oogamous and takes place by the fusion of a small biciliate motile antherozoid with a large non-ciliated egg, which escapes out of its parent wall. This appears to be the first record of oogamous reproduction in the genus *Carteria*.

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Names of genera and species should be underlined and will appear in italics. The names of the authors of genera and species should always be given.

Original papers must conclude with a summary, drawing attention to the main facts and conclusions. References to literature cited should, as far as possible, be complete and must be carefully verified. A bibliography should be given at the end of the paper arranged alphabetically under authors' names.

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A CONTRIBUTION TO THE LIFE-HISTORY OF *DESMODIUM GANGETICUM* DC.

By J. V. PANTULU

Maharaja's College, Vizianagram

(Communicated by A. C. Joshi)

CYTOLOGICAL work on the genus *Desmodium* is limited to observations on the chromosome numbers of four species. Kawakami (1930) reported 11 n chromosomes in *Desmodium perpesium* DC. Cooper (1936) found the same number in *D. grandiflorum* (Walt.) DC. Recently Senn (1938) noted 11 n chromosomes in *D. canadense* (L.) DC. and *D. tortuosum* DC. There is no previous work on the embryology of this genus. During the present investigation I have studied the structure and development of the anther, pollen, ovule and embryo-sac of *Desmodium gangeticum* DC. The number of meiotic chromosomes is also reported.

Desmodium gangeticum is a widely distributed species, being found throughout India, Ceylon, Burma, S.E. Tropical Asia, China, Philippines and Trop. Africa. It is a slender undershrub, usually 2–4 ft. high. It grows abundantly near Benares and all over the United Provinces under the shade of other trees. The white-lilac flowers are borne in 6–12″ long racemes and appear during the rainy season. The plants are employed in Indian medicine.

The material was collected from Benares during the month of September 1939, between the hours 12 noon and 3 P.M. on sunny days. It was fixed in Navashin's fluid for 16-20 hours, then rapidly rinsed in water 4 or 5 times and transferred to 70 % alcohol. Further dehydration of the material and embedding in paraffin was carried out according to the customary methods. The sections were cut 8–12 μ thick. Slides intended for chromosome study were stained with Newton's Iodine Gentian-violet (Crystal-violet was used instead of Gentian-violet). For morphological work the material was stained with Delafield's Hæmatoxylin.

DEVELOPMENT OF THE ANTHER AND POLLEN

The different floral whorls differentiate in acropetal order.
The early development of the anther is quite normal. At first it is
quite cylindrical and consists of homogenous cells, but as the
primary archesporium differentiates the anther becomes 4-lobed.
The primary archesporium is limited to one row of hypodermal cells
in each lobe (Fig. 1). These cells divide periclinally to form the
primary wall cells to the outside and primary sporogenous cells on
the inside (Fig. 2). The former by further divisions give rise to four
layers of parietal cells (Figs. 3 and 4), of which the innermost
develops into the tepetum, the outermost into the fibrous endo-
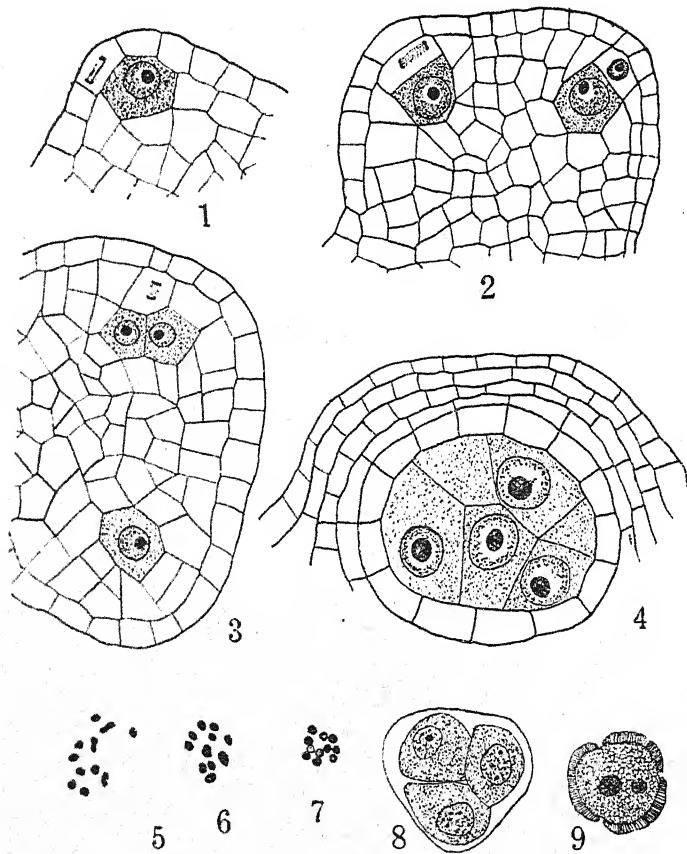
Figs. 1-9. *Desmodium gangeticum.*—Figs. 1-4. Transverse sections
of anther-lobes at various stages of development showing the differentiation
of the sporogenous and parietal tissues. Figs. 5-7. I meiotic division
metaphase plates showing 11 bivalents. Fig. 8. A tetrahedral tetrad of
pollen grains. Fig. 9. A mature pollen grain. Figs. 5-7, × 1200; the
rest, × 750.

thecium. The two middle layers are crushed during the further development.

The tapetum is of the secretion type. The tapetal cells considerably increase in size after their differentiation and develop conspicuous vacuoles, but they always remain at the periphery of the pollen-sac. Generally among angiosperms the nucleus of the tapetal cells undergoes one mitotic division at the time of I meiotic division in pollen mother cells and the tapetal cells become binucleate. In *Desmodium gangeticum*, however, no such division occurs and the tapetal cells remain uninucleate throughout their life. The same feature has been observed previously in *Phaseolus vulgaris* (Weinstein, 1926), *Lathyrus odoratus* (Latter, 1926), *Medicago sativa* (Reeves, 1930 ; Cooper, 1933) and *Melilotus alba* (Castetter, 1925). I have also seen permanently uninucleate tapetal cells in *Tephrosia purpurea* and *Cyamopsis psoralioides*, two other members of the family. Uninucleate tapetal cells, therefore, appear to be a common feature of the Papilionaceæ.

The primary sporogenous cells undergo approximately two more mitotic divisions to develop into pollen-mother cells. These show the usual characters. The two meiotic divisions proceed normally. At the I meiotic metaphase 11 bivalents are clearly seen (Figs. 5–7). The number of chromosomes in *Desmodium gangeticum*, therefore, is the same as in *D. perpesium* (Kawakami, 1930), *D. grandiflorum* (Cooper, 1936), *D. canadense* and *D. tortuosum* (Senn, 1938). This number is comparatively rare in the Leguminosæ, but all investigated species of the genus *Desmodium* have been found to show the same number of chromosomes. Cytokinesis takes place by furrowing. The pollen tetrads are mostly of the tetrahedral type (Fig. 8). After their liberation from the mother cell the young pollen grains develop the usual exine and intine. Their nucleus also divides once. The pollen grains are shed at the 2-nucleate stage (Fig. 9). They show three germinal furrows. These characters are common to many Leguminosæ (Wodehouse, 1935 ; Schnarf, 1939).

OVULE AND THE EMBRYO-SAC

The carpel is the last part to differentiate in the flower. The carpel primordium is notable for the fact that the cells of the dorsal suture and its vicinity lose their meristematic character much earlier than the cells of the carpel margins. The ovules arise as the result of the activity of groups of hypodermal cells on the carpel margins. This is at a stage before the carpel margins have met and fused. The carpel is still open at the ventral suture. There are approximately 8 ovules in a carpel of *Desmodium gangeticum* arranged in one longitudinal row, the alternating ones coming from different carpel margins. The ovule primordia inside the ovary grow at first quite straight towards the dorsal suture, but even before the integument initials appear, they curve upwards due to greater growth on the lower side. Reeves (1930) in *Medicago sativa* noted that the
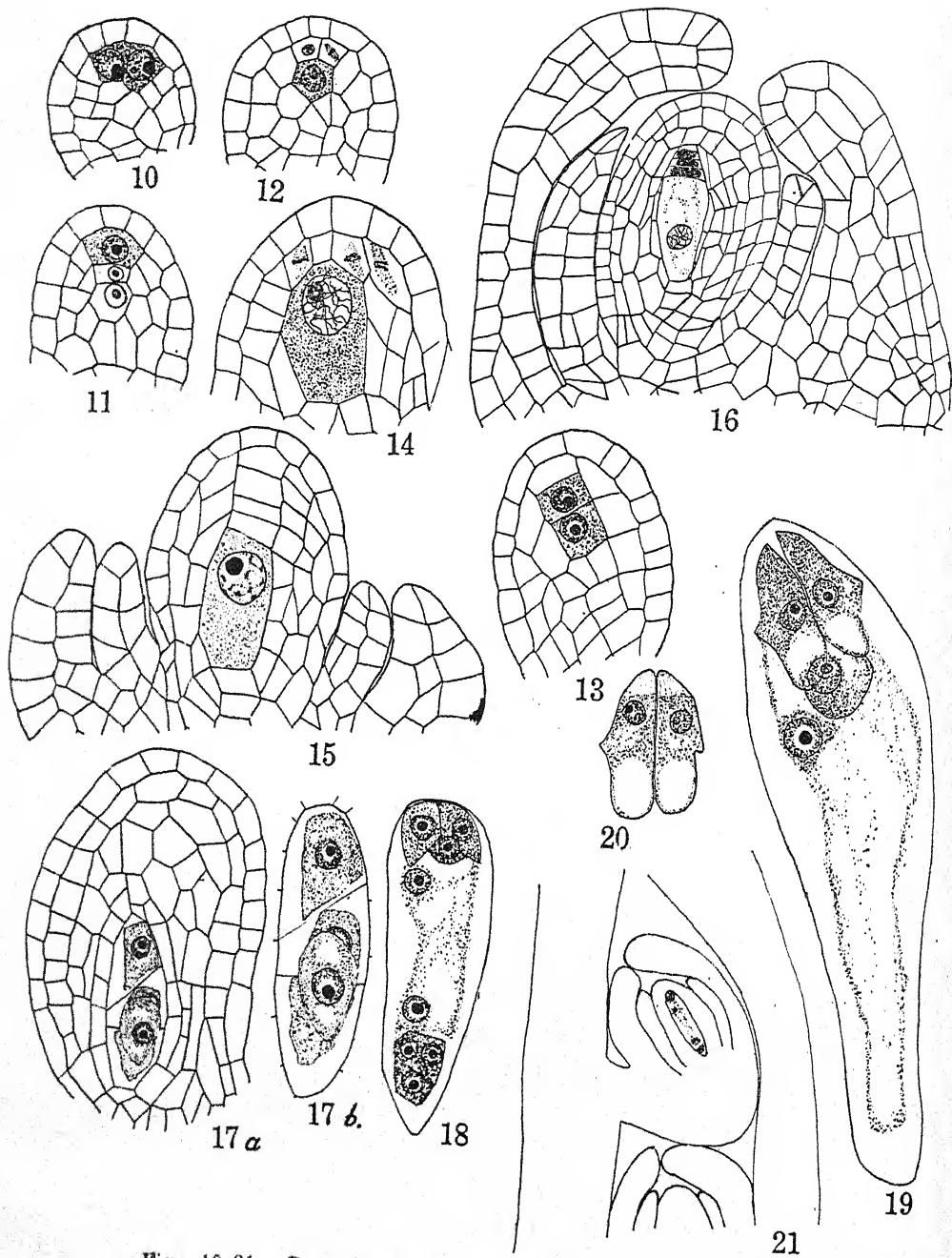
Figs. 10–21.　*Desmodium gangeticum.*—Figs. 10–11. Nucellus showing primary archesporium. Figs. 12–15.　Nucellus with megaspore mother

curvature of the ovules is due to mechanical causes. He finds that the ovules grow quite straight till they meet the dorsal wall of the carpel. Then their straight growth is checked and they curve upwards or downwards. No such relation has been seen in *Desmodium gangeticum*. The ovules begin to curve upwards (towards the side of the stigma) even before they have come in contact with the dorsal wall of the carpel. The mature ovule has a nearly ana-amphitropous form (Fig. 21).

The primordia of the integuments appear simultaneously with the formation of the megaspore-mother cell. That of the inner integument appears slightly before that of the outer. Both the integuments are at first two cells thick, but the outer integument later becomes thicker, particularly near the micropyle. At the time of fertilisation through its greater length it consists of 3-4 layers of cells, while near the micropyle it is 5-6 cells thick. The inner integument near the micropyle is also 3-4 cells thick. The outer integument, even though it begins to develop after the inner, soon outgrows the latter, and alone forms the micropyle. This is a very general feature in the Leguminosæ. Roy (1933), however, has observed an exception to this condition in *Cajanus indicus* and *Lathyrus sativus*. He finds in these species that the inner integument is not only much less developed than the outer, but even its primordium differentiates after that of the outer integument. Newman (1933) has reported that in *Acacia Baileyana* the integuments are poorly developed. The ovule consequently is almost naked.

There is generally a single primary archesporial cell in the ovules of *Desmodium gangeticum*, but before the formation of the primary wall cell, it is not possible to distinguish this cell from the immediately surrounding ones. As the primordia of the ovules begin to curve upwards, we find the hypodermal cells at the apex of the nucellus, numbering 3–5, increase in size and begin to stain differently from the other cells of the nucellus (Fig. 10). They also possess larger nuclei and denser cytoplasm as compared with the other cells. Then just one of these cells divides by a periclinal wall into a primary wall cell and the megaspore-mother cell (Fig. 12). This cell may be said to function as the primary archesporial cell, though potentially all the hypodermal cells at the apex of the nucellus appear to be archesporial. I do not think it proper to call this condition as multi-cellular archesporium, though previously multicellular archesporium has been noted in a number of Papilionaceæ,—*Vicia* and *Trifolium* (Martin, 1914), *Phaseolus* (Brown,

cell. Fig. 16. An ovule showing linear tetrad of megaspores, of which the three micropylar megaspores are degenerating. Fig. 17*a*. A nucellus with an ' inverted T-shaped ' tetrad of megaspores. Fig. 17*b*. The tetrad seen in 17*a* is shown on a larger scale. Fig. 18. A young 8-nucleate embryo-sac. Fig. 19. Mature embryo-sac. The antipodals have degenerated and the polar nuclei have fused. Fig. 20. Synergidæ from another embryo-sac showing hooks and ' filiform-apparatus '. Fig. 21. Apical part of a longitudinal section of the ovary showing an ovule at the 8-nucleate embryo-sac-stage. Fig. 16, × 360 ; Fig. 17*b*. × 1200 ; Fig. 21, × 150 ; the rest, × 750.

1917), and *Medicago* (Reeves, 1930).   In one case a sub-hypodermal cell also has been seen to have all the characters of the archesporium (Fig. 13).   Often a row of two or three ' supporting cells ' is seen below the megaspore-mother cell, but this may be distinct even from the archesporial stage (Fig. 11).   Perhaps such a row arises from the division of ' archesporial-like ' sub-hypodermal cell shown in Fig. 13.

The meiotic divisions in the megaspore-mother cell take place in the normal manner.   At the end of the first division a transverse wall is laid, so that two dyad cells are organized, of which the micropylar is smaller than chalazal.   This is due to the position of the nucleus of the megaspore-mother cell.   This is always situated near the micropylar end of the megaspore-mother cell.   At the end of second meiotic division again two transverse walls are formed, so that a linear tetrad of megaspores results (Fig. 16).   In one exceptional case, however, after the second meiotic division the chalazal dyad was found to have divided by a longitudinal wall.   Thus an ' inverted T-shaped tetrad ' had resulted (Fig. 17*a* and *b*).   This type of tetrad is known in several Onagraceæ, where the micropylar megaspore is the functional one and the embryo-sac is of the *Oenothera*-type (monosporic 4-nucleate).   It was observed by Johansen (1931*a* and *b*) in *Anogra pallida* and *Zauschneria latifolia* for the first time.   Later Maheshwari and Gupta (1934) reported the occurrence of ⊥-shaped tetrad in *Ludwigia parviflora*.   Last year, Kajale (1940) reported one instance of this type of tetrad in *Cyathula tomentosa* (Amarantaceæ), a form possessing normal type of embryo-sac.   Three other instances are mentioned by Maheshwari (1941), namely *Drimiopsis maculata* investigated by Baranow, *Tacca viridis* studied by Paetow and *Styrax officinalis* recently investigated by Copeland.

In the Leguminosæ in some species the chalazal megaspore is the functional one ; in others it is the one next to it (Guignard, 1881). Variation is found within the same genus, *e.g.*, *Cassia* (see Summary of literature by Datta, 1935) ; sometimes even within the same species, *e.g.*, *Albizzia Lebbek* (Maheshwari, 1931).   In *Desmodium gangeticum*, however, always the chalazal megaspore was found to develop into the embryo-sac.   The three micropylar megaspores degenerate, though their traces are visible up to the 2-nucleate stage of the embryo-sac.   In the case of the ovule with ⊥-shaped tetrad of megaspores, it appears probable that one of the two chalazal megaspores develops into the embryo-sac.   As seen in Fig.17, it has considerably increased in size and developed the polar vacuoles to form a uninucleate embryo-sac.   Further development proceeds according to the *Normal*-type and results in the formation of an 8-nucleate embryo-sac (Fig. 18).

Of the three cells of the egg-apparatus, the egg is slightly larger than the synergidæ.   The egg has a large vacuole so that the cytoplasm and the nucleus are pressed towards the chalazal end.   The egg nucleus is larger than those of the synergidæ (Fig. 19).   The synergidæ (Figs. 19 and 20) have a large vacuole in the chalazal

half. The cytoplasm is mostly found in the micropylar half. The nucleus is situated just above the vacuole. The synergidæ are prominently hooked and in the advanced stage clearly show the ' filiform apparatus '. The antipodals degenerate early. This condition has been reported in many other Papilionaceæ and seems to be characteristic of the family. Both the polar nuclei move to the centre of the embryo-sac and there they fuse at an early stage. The secondary nucleus then moves to the neighbourhood of the egg-apparatus and remains there till the time of fertilisation.

The mature embryo-sac appears nearly lanceolate in longitudinal section at the time of fertilisation (Fig. 19). The broad end faces the micropyle of the ovule. During its growth it destroys almost the whole of the nucellus, so that the micropylar end of the embryo-sac at the fertilisation stage is covered only by one layer of cells, the epidermis of the nucellus. This feature is also seen in many other Papilionaceæ.

SUMMARY

The development and structure of the anther, pollen, ovule and embryo-sac and the number of chromosomes in *Desmodium gangeticum* DC. have been investigated.

The primary archesporium in each anther-lobe is limited to one hypodermal row of cells. The tapetum is of parietal origin and of the secretion type. The tapetal cells remain 1-nucleate. Mature pollen grains are 2-nucleate and possess 3 germinal furrows. The haploid number of chromosomes is 11.

The ovules are ana-amphitropous with the micropyle pointing upwards. The latter is formed only by the outer integument. There is generally a single archesporial cell, which cuts off a parietal cell.

The parietal tissue is rather poorly developed. It consists of 3-4 layers in the early stages, but is all destroyed by the growth of the embryo-sac, so that at the time of fertilisation the embryo-sac at the micropylar end is covered only by the epidermis of the nucellus.

The megaspore-mother cell gives rise to a linear tetrad of megaspores, but in one instance an ' inverted T-shaped ' tetrad was seen. The chalazal megaspore develops into the embryo-sac according to the *Normal*-type. The synergidæ are hooked and possess the ' filiform apparatus '. The antipodals degenerate early.

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STUDIES IN THE PROTEACEÆ

VI. Structure and Development of the Seedling of *Grevillea robusta* Cunn.

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(Communicated by P. Maheshwari)

Bancroft (1930), in discussing the arborescent habit in angiosperms, gives a review of seedling structure in the different families. No reference to the Proteaceæ is found in this review, and there seems to be no work on the seedling structure of this family apart from a very general account of the external morphology given by Lubbock in 1892. The present work on *Grevillea robusta* Cunn. was, therefore, undertaken to fill this gap at least partly.

Material and Methods

The seeds were collected locally from several plants. Some were sown in ordinary garden soil under greenhouse conditions, while others were germinated in sterilized saw dust in the laboratory. In both cases the germination was satisfactory. The seedlings grown in saw dust were mainly used for observing the germination stages, as well as for preparing serial microtome sections. The seedlings raised in garden soil were used for checking the results so obtained.

The material for sectioning was killed in Bouin's fluid and some was also preserved in formalin-acetic acid-alcohol. Serial transverse and longitudinal sections, cut at various selected levels in the seedling, were used to study the vascular structure ; many freehand sections were also examined. Microtome sections were cut from 10 to 15 μ in thickness and were all stained in alcoholic safranin ; for contrast some sections were counterstained with light green dissolved in clove oil.

Germination and Development of the Seedling

The seed of *Grevillea robusta* is thin and flat and contains a large embryo with two cotyledons each of which is provided with two basal lobes, the *auricles* (Fig. 1). The time required for germination is a long one, about a fortnight. During this period, the seed swells somewhat and the seed-coat becomes slightly softened. Gradually the radicle curves slightly towards one side of the seed, usually the dorsal (Fig. 2), and by further growth forces open the seed-coat

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along the edge (Fig. 3).   At this time the hypocotyl shows a knob-like swelling at the base which persists in the developing seedling (Figs. 4, 7–11).   This swelling (which may not, however, be equally pronounced in all the seedlings) plays an important rôle in forcing apart the seed-coat into two flaps extending from the micropyle downwards (Figs. 12 *a*, 12 *b*).   The part of the hypocotyl above the basal swelling now begins to elongate rapidly and at the same time forms an arch at the top (Fig. 7).   The basal portion of the hypocotyl suddenly slips out of the seed-coat (Fig. 4) and the subsequent
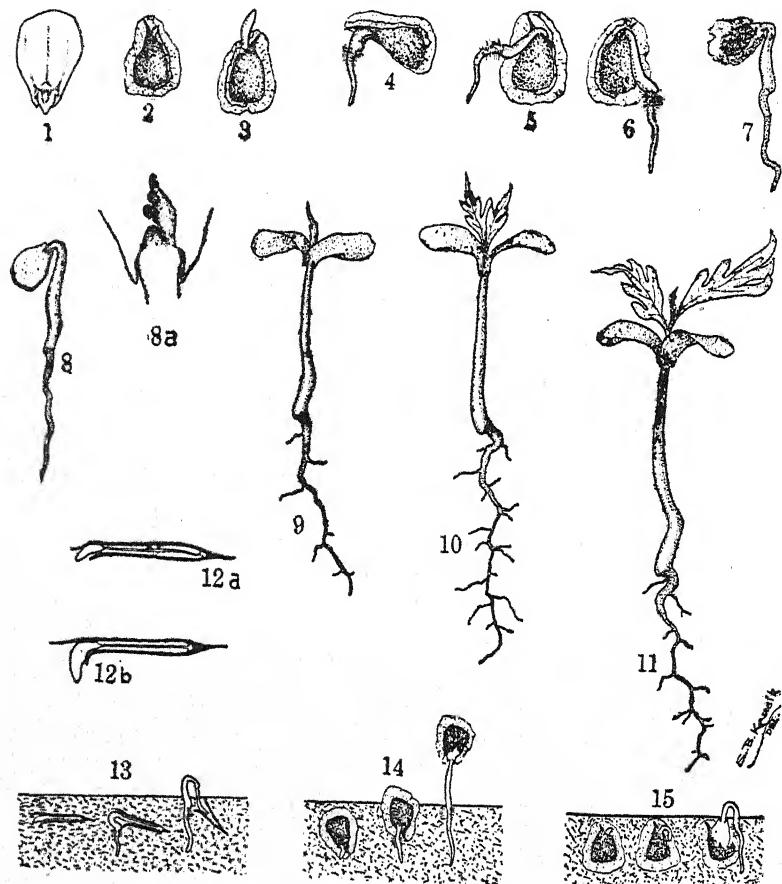
Figs. 1–15.   Fig. 1.   Embryo dissected out of the seed showing the cotyledon (the other cotyledon is removed) with its basal lobes.   × 1·5. Figs. 2–11.   Stages in the development of the seedling.   All × 0·75 except 8 *a* about × 4.   In Figs. 4 and 7–11 the base of the hypocotyl shows the cortical swelling ; the young epicotyledonary shoot is shown in Fig. 8 *a*. Figs. 12 *a* and 12 *b*.   Diagrams of seed showing the formation of the basal swelling of hypocotyl.   Figs. 13–15.   Diagrams of seeds germinating in different positions in the soil.

growth of the hypocotyl takes place mainly between the basal swelling and the upper arch. The growing seedling thereby gradually brings the cotyledons, still held within the seed-coat, very near the surface of the soil (Fig. 13). After this the arch straightens and the cotyledons are pulled above the soil with the seed-coat left behind. In some cases, when the seed is sown in the soil in a vertical position with the micropyle pointing downwards, the cotyledons, still retained within the seed-coat, appear above the soil at the end of a more or less upright hypocotyl in which the arch is apparently not formed at all previously (Fig. 14).

Soon after the cotyledons appear above the ground after discarding the seed-coat, they become deep green in colour and diverge from the node. The young epicotyledonary shoot, which at first lies concealed between the two cotyledons (Fig. 8 *a*), begins to grow rapidly. The leaves of this shoot arise according to a two-fifths spiral phyllotaxy.

The knob-like swelling at the base of the hypocotyl consists of radially elongated cortical cells and is evidently a localized cortical growth. It attains its maximum dimensions in some cases when the seeds germinate in a more or less flat position (Fig. 12 *a*, 12 *b*, 13), while in other cases, when the seeds are sown in any other position (Figs. 14, 15), it is considerably reduced in size or may even be absent. Further, its formation depends largely on the nature and the direction of stimuli operating during germination and the development of the seedling.

This basal swelling of the hypocotyl appears to have the same rôle as that of the " peg " in the Cucurbitaceæ. In *Citrullus vulgaris* Shrad., Hufford (1938) states that the formation of the peg is rather variable, depending on the position in which the seeds germinate, and that it is a " cortical structure developed in accordance with the direction of the stimuli which initiate it ".

Another interesting feature of the seedling of *Grevillea robusta* is that the cotyledons often persist even till very late stages. They were seen in seedlings which were as old as six months, though, they were almost in a wrinkled state and about to fall away.

The Primary Root

The primary root has a tetrarch radial vascular cylinder with a large pith in the centre (Fig. 16 *g*). In transverse sections the four groups of centripetally developing xylem are seen arranged diagonally with reference to the cotyledonary and the intercotyledonary planes, and the phloem groups occupy the alternating radii (Fig. 21). In early stages of root development, the phloem consists merely of uniform parenchymatous cells, but later the differentiation into the sieve tubes and the companion cells becomes evident. Outside the alternating groups of xylem and phloem, there is a two to three layered pericycle which is surrounded by the endodermis. The latter is easily recognisable by the presence of the characteristic Casparian thickenings on the radial walls of its

2*a*
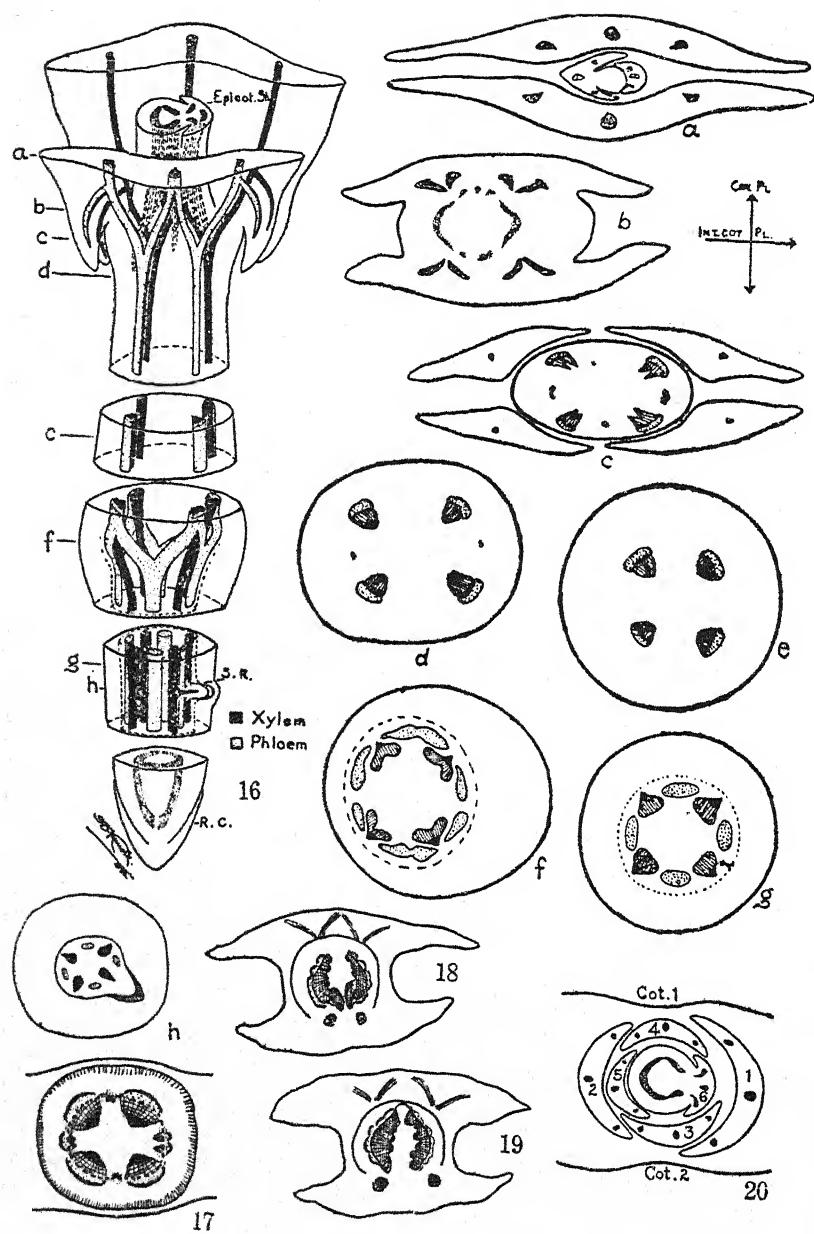
Text-figs. 16–20.—Fig. 16. A diagrammatic drawing of the seedling to show the courses of the vascular strands in the different parts. Fig. 16 *a-h*. Diagrams of transverse sections of seedling at various levels corresponding to the markings in Fig. 16. Fig. 17. Diagram of transverse section of

cells (Fig. 21). The cortex consists of about eight to ten layers of cells surrounded externally by the epidermis. As the root grows older the outer cortical layers become suberized and slough away.

In a longitudinal section of the root, the apex is occupied by a common meristem from which all the cortical and stelar tissues are formed (Fig. 22). This meristem lies in a horizontal plane and has the appearance of a very shallow bowl with the sides sloping upwards gently. Cells are cut off to the inside, as well as to the outside by this meristem. Of the cells cut off towards the inside, those that are formed from the bottom of the bowl give rise to the central cylinder, while the others formed from the sloping sides contribute to the formation of the cortex and epidermis. The epidermis and the regularly arising rows of cortical cells can be easily followed from the meristem in proper median sections. The cells that are formed towards the outside of the meristem give rise to the root-cap in which the arrangement of cells is seen to be somewhat regular only for a short distance from the meristem, while it is very irregular at the tip of the root (Fig. 22).

DEVELOPMENT OF THE SECONDARY ROOT

The secondary root develops opposite one of the xylem points of the primary root (Figs. 16 *h*, 24) and shows the same organization of tissues. In the formation of the secondary root some of the cells of the pericycle opposite the protoxylem begin to divide tangentially (Fig. 23) to form several layers of regularly arranged cells, which further undergo radial divisions. The cells so formed constitute the initials of the secondary root in which the differentiation into the central cylinder with rows of elongated cells, the cortex, and the epidermis becomes very soon evident (Fig. 24). In the meanwhile, the cells of the endodermis in the primary root also divide in both tangential and radial planes to form a series of cells which constitutes a " temporary " root-cap for the secondary root. An apical meristem is formed in the young secondary root in the same manner as in the mother root and further growth takes place by the activity of this meristem (Fig. 24). The secondary root grows in length by penetrating through the cortex of the primary root and finally emerges out by rupturing the epidermis.

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hypocotyl at the cotyledonary node to show the formation of secondary structures in the hypocotyl strands. Figs. 18 & 19. Diagrams of transverse sections of the cotyledonary node at two slightly different levels to show the two large arcs of epicotyledonary vascular system lying in the intercotyledonary plane. Fig. 20. Plan of the epicotyledonary shoot to show the sequence of the leaves in a two-fifths spiral phyllotaxy and the formation of leaf gaps and leaf traces. In all the figures the vascular strands are shown in the different parts of the seedling in the same positions with reference to the cotyledonary and the intercotyledonary planes as indicated by the cross at the right-hand top corner of page.

Cot. 1. Cotyledon 1; *Cot.* 2, Cotyledon 2; *Cot. Pl.*, Cotyledonary plane ; *Int. Cot. Pl.*, Intercotyledonary plane ; *Epicot. sh.*, Epicotyledonary shoot ; *R.C.*, Root-cap ; and *S.R.*, Secondary root.
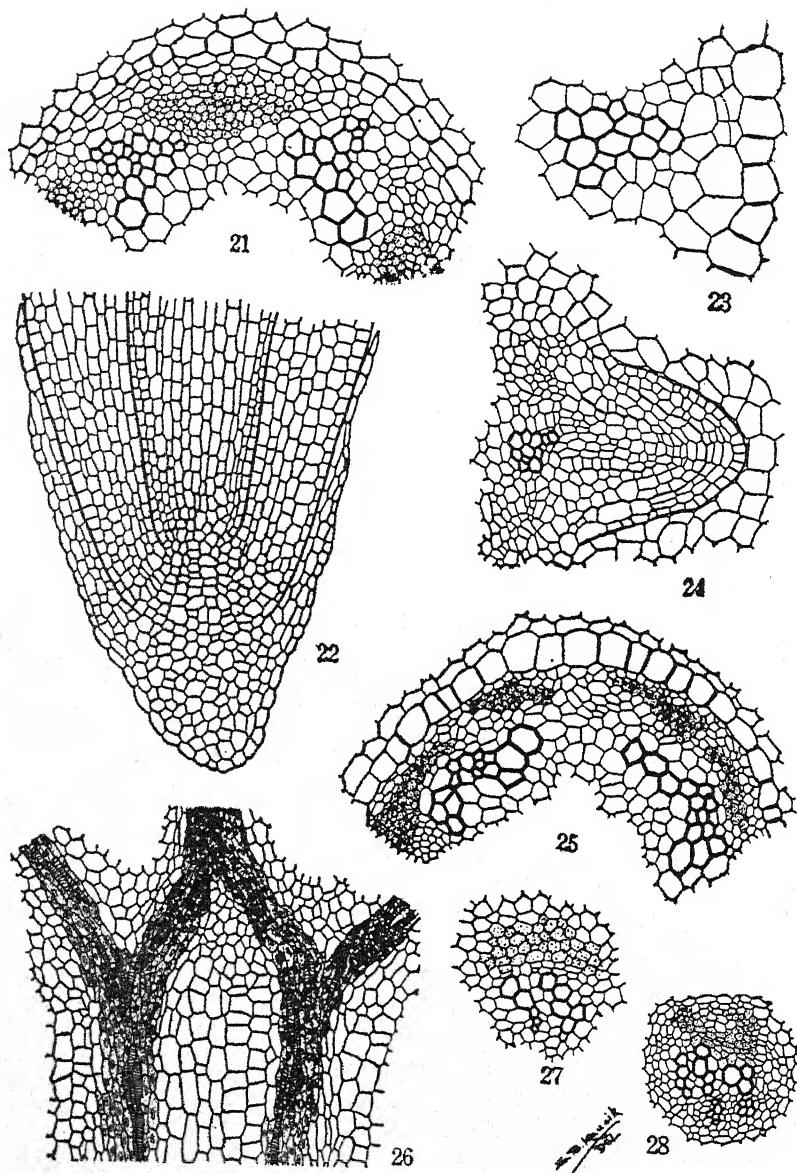
Figs. 21–28.—Fig. 21. Part of transverse section of young root showing two of the four diagonal xylem groups and the alternating groups of phloem. Fig. 22. Longitudinal section of the root tip showing the root-cap and the origin of the cortical and stelar tissues from a common meristem. Fig. 23. Some of the pericycle cells opposite the xylem of the primary root showing tangential divisions to form the secondary root. Fig. 24. The secondary root about to penetrate through the cortex of the

The Transition Region

The changes involved in the transition from root to stem structure are clearly seen in transverse sections at the base of the hypocotyl where the knob-like swelling develops (Figs. 16, 25). This region of transition is a fairly short one and constitutes the so-called "collet". The four groups of xylem and phloem are subjected here to certain changes which involve splitting, fusion, and reorientation to give rise to the collateral endarch condition in the stem. During transition, the xylem strands do not split, for, at no stage is there seen an actual doubling of the number of xylem groups. On the other hand, each group assumes a "V"-shaped appearance in transverse sections as soon as the transition level is reached, the metaxylem forming the two limbs of the letter and the protoxylem occupying the apex. At a slightly higher level, the two metaxylem arms swing tangentially towards the outside (Fig. 25), and at a still higher level are seen approaching each other. Finally the two arms fuse together outside the protoxylem to give rise to the typical endarch condition. The xylem strands thus occupy the same diagonal planes in the hypocotyl after transition.

While the movements of the metaxylem arms are taking place, the groups of phloem also undergo certain changes. Each phloem group of the root splits into two almost equal halves in the region of transition (Figs. 16 *f*, 25) so that transverse sections here show twice the number of phloem groups found in the root. These groups sometimes lie so close to one another that transverse sections at particular levels in the transition region may show an almost continuous ring of phloem. At slightly higher levels, the halves belonging to the original adjacent groups of phloem begin to move laterally towards each other so that they form pairs, and finally fuse together outside the metaxylem when the transition is completed. The change from the radial alignment in the root to the collateral alignment in the stem is effected in this way.

The mode of root-stem transition described here corresponds to the third type of van Tieghem (1891). According to this type, the number of vascular bundles in both the hypocotyl and the root is regarded to be the same, transition taking place by splitting and subsequent fusion in pairs of the phloem groups. In addition to the three types of transition first recognized by van Tieghem, Sargant

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primary and showing the details of structure. Fig. 25. Part of transverse section of hypocotyl at the transition region showing splitting and lateral movements of phloem groups, the xylem groups becoming "V"-shaped. Fig. 26. Part of longitudinal section of hypocotyl near the cotyledonary node to show forking of hypocotyl strands and the subsequent fusion of the inner limbs to form the midrib strand of the cotyledon, the outer limbs forming the marginal strands. Fig. 27. One of the hypocotyl strands just below the cotyledonary node to show the presence of cambiform cells. Fig. 28. The midrib strand of the cotyledon soon after leaving the cotyledonary node. All figures × 200, except Fig. 23. × 400 and Fig. 26, × 120.

(1900) found a very rare fourth type. All these types have been mentioned by Eames and MacDaniels (1925).

THE HYPOCOTYL

The hypocotyl has four diagonally arranged vascular strands which are collateral endarch (Fig. 16 *d*, *e*). These strands run without any change throughout the length of the hypocotyl. Towards the upper portion of the hypocotyl, especially near the cotyledonary node, these diagonal strands are not equally spaced but form two recognizable pairs, each pair being in relation to a cotyledon (Fig. 16 *c*, *d*). There is slightly more space betweeen the members of the two pairs than between the pairs themselves. This is evidently associated with a slight flattening of the hypocotyl at this region. The cotyledonary node of the hypocotyl is also interesting in showing anastomoses of the vascular strands. Before the formation of such anastomoses, the members of each pair of the hypocotyl strands fork into two limbs, so that two rows of four bundles each are seen towards the two flattened sides of the hypocotyl in transverse sections at this region (Fig. 16 *b*). The two median bundles of each row, representing the limbs formed towards the inside by one pair of hypocotyl strands, gradually meet together at slightly higher levels and finally fuse together to give rise to a single large strand (Fig. 26). This strand next enters the cotyledon on the corresponding side as its midrib strand. The two outer bundles of the row, namely the outer limbs formed by the hypocotyl strands, move out at the cotyledonary node and enter the cotyledon to form its two marginal strands (Fig. 26).

The hypocotyl strands at the cotyledonary node display yet another interesting feature ; cambiform cells are seen here between the xylem and phloem (Fig. 27) even in young seedlings. This indicates that secondary activity begins in the hypocotyl strands very much earlier at this region than elsewhere below the cotyledonary node. The significance of this becomes apparent when one considers that the vascular cylinder of the young epicotyledonary shoot, first arising in the form of groups of provascular tissue, become connected to the hypocotyl strands only after secondary vascular elements are formed at the cotyledonary node.

The cortex of the hypocotyl in a young seedling does not show any differentiation. It is composed of uniform parenchymatous cells, the outer layers containing chlorophyll. In older seedlings the outer layers become suberized and are peeled off along with the epidermis (Fig. 34). In these the vascular strands show a considerable development of secondary structures, and also the formation of sclerenchymatous fibres by the primary, as well as a part of the secondary phloem (Fig. 35).

THE COTYLEDONS

After the formation of the anastomoses between the limbs of the hypocotyl strands at the cotyledonary node, the main vascular

supplies to the two cotyledons, consisting in each case of a midrib strand and two marginal strands, can be easily made out (Figs. 16 *a*, 26). The midrib strand, being formed by the fusion of the two inner limbs of the hypocotyl strands, appears in transverse sections of the cotyledon almost twice as large as the marginal strands. Its double nature is very evident at the base of the cotyledon (Fig. 28), while higher up, its two components may practically be so intimately associated together that its larger size alone must be suggestive of this fact. The three main strands of the cotyledon form numerous branches which ramify in the tissues of the cotyledon. In addition, each marginal strand gives rise to a large branch at the base of the cotyledon which immediately curves down sharply and enters the basal lobe of the cotyledon on the corresponding side (Fig. 16, 16 *c*).
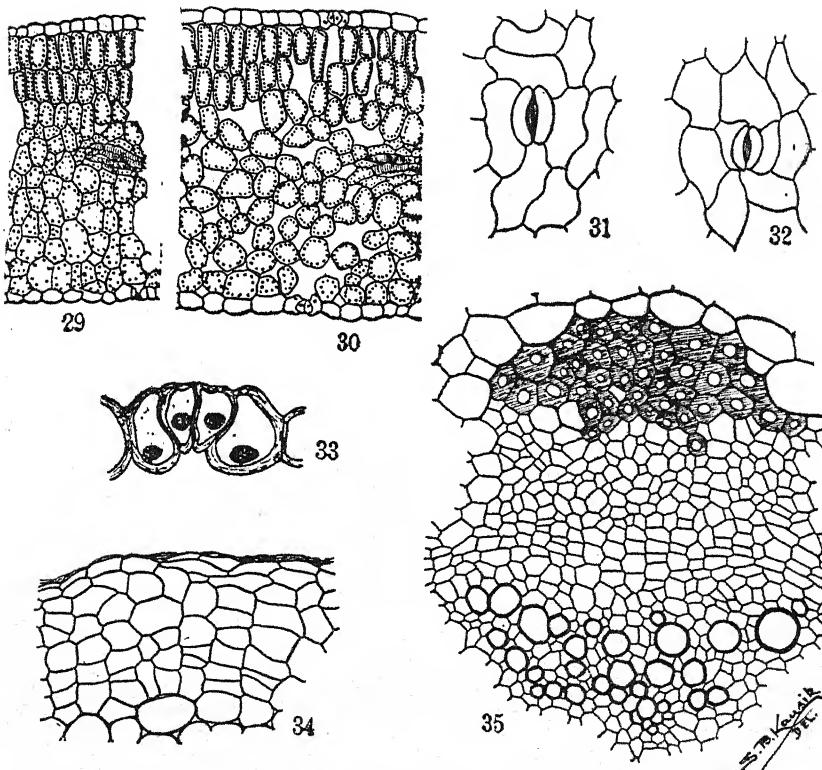
Figs. 29–35.—Figs. 29 & 30. Sections of cotyledon at two stages in the differentiation of its mesophyll. × 120. Figs. 31 & 32. Parts of lower and upper epidermis respectively to show the arrangement of cells and the stoma. × 200. Fig. 33. A stoma of the lower epidermis in section. × 600. Fig. 34. Outer layers of cells of the hypocotyl in an old seedling. × 200. Fig. 35. A vascular strand of the hypocotyl of an old seedling showing the development of secondary structures; the sclerenchymatous fibres are formed by the phloem. × 400.

As soon as the cotyledons become epigeal they appear quite green in colour. At first there is only a slight differentiation of the mesophyll into the palisade and spongy tissues, the cells in both being rather compactly arranged (Fig. 29). In slightly older cotyledons the differentiation becomes very marked and large air spaces are seen in the spongy tissue (Fig. 30). Some smaller air spaces are also seen here and there in the palisade layer, especially near the stomata. The palisade tissue has two layers of columnar cells, while the spongy region shows many more layers.

The cells of both the upper and the lower epidermis are irregular in outline presenting a mosaic-like appearance (Figs. 31, 32). Stomata are present in both the layers, but are more numerous per unit area, also slightly larger, in the lower than in the upper epidermis (Figs. 31, 32).

The Epicotyl

No extensive studies of the epicotyledonary shoot were made other than merely to establish the nature of its vascular connections at the cotyledonary node. The epicotyledonary shoot begins to grow rapidly as soon as the cotyledons leave the seed-coat and appear above the surface of the soil. The primary vascular structures of this shoot first arise in the form of distinct groups of provascular tissues which descend into the cotyledonary node. These groups are not continuous with the hypocotyl strands, but lie between them with some amount of parenchyma intervening. Two of these groups are much larger than the other two and are found in the form of two arcs, one on each side of the hypocotyl in the intercotyledonary plane (Fig. 16 b, c). The other smaller groups are found towards the flattened sides of the hypocotyl in the cotyledonary plane (Fig. 16 b, c). In older seedlings these groups of epicotyledonary vascular tissue become connected to the four hypocotyl strands when secondary vascular structures are formed at the cotyledonary node. As secondary growth proceeds further, the vascular cylinder of the epicotyledonary shoot is seen in the form of two semicircular arcs on either side in the intercotyledonary plane (Figs. 18, 19). Higher up in the shoot this cylinder becomes ring-like and at a still higher level it has a horse-shoe appearance on account of a large gap formed in connection with the vascular supply to the first leaf (Fig. 20). Soon after this the gap again closes, but reappears at every succeeding node when a leaf is formed according to a two-fifths spiral phyllotaxy (Fig. 20).

The arrangement and the course of the vascular strands in the different parts of the seedling and also the relationship of the epicotyledonary vascular tissue to the hypocotyl strands are shown in a diagrammatic form in Fig. 16. Outlines of transverse sections of the seedling at various selected levels are shown in Fig. 16 a to h marked correspondingly in Fig. 16.

Conclusions

Thomas (1907) discusses the nature of the median strand of the cotyledon in angiosperms and points out that it is double, occurring

either as too widely separated entities, or "so closely approximated as to have the appearance of a single strand" (cited from Kimmell, 1936). In *Grevillea robusta* the median strand appears to be single, but is nevertheless twice as large as the two marginal strands of the cotyledon. Its double nature is evident at the base of the cotyledon, and still lower, at the cotyledonary node, the approximation together and fusion of two bundles of the hypocotyl strands can be easily followed. Further, the anastomoses of the hypocotyl strands at the cotyledonary node seem to play an important part in the formation of the main vascular supplies of the cotyledons.

Bancroft (1930) states that from the extensive work of Miles Thomas (1923) "it appears that the variations in seedling anatomy are not due to difference in basal plan, but to the varying behaviour of a common fundamental unit of vascular structure and its associated strands". Despite this many modifications of the vascular strands are met with in the cotyledons and the hypocotyl; from the different behaviour of these strands at various levels below the cotyledonary node certain types of root structure result. These types are referable to the *cruciform* and the *diagonal* plans. The former type is met with very commonly, while the latter type occurs only in a very few cases. Important modifications of both the types occur depending on the number of root poles. Davey (1916) has shown that both the *cruciform* and the *diagonal* types are found in the several groups of the Amentiferæ, of which the Fagales show a wide range of root structure and also transition from one type to the other. In the order Ebenales, again, both the types occur according to Miles Thomas (1923), diagonal tetrarchy being found in the Sapotaceæ and in some species of *Diospyros* belonging to the Ebenaceæ. In the Ranales the root symmetry is diagonal tetrarch in the Calycanthaceæ only.

Holden and Bexon (1923) find that a lateral concentration of the strands of the cotyledons takes place at the cotyledonary node in *Acer pseudoplatanus* and state that as a consequence of this the hypocotyl shows a diagonal arrangement. They further remark that this arrangement continues throughout the hypocotyl and that a cruciform condition results only when the vascular strands enter the root. They regard the diagonal arrangement to be an evolved condition from an original cruciform one, and suggest that the seedling structure in *Acer* is only a preliminary stage which culminates in the final establishment of a diagonal root symmetry seen in certain cases in some angiosperms. Holden and Clarke (1926) remark that variations in hypocotyl structure are due in great measure to "linkage, fusion or independence" of the components of the cotyledonary vascular system.

In the case of *Grevillea robusta* the main vascular system of the cotyledons is so related to the cotyledonary node that anastomoses occur here and the hypocotyl shows a diagonal arrangement which continues down to the base of the hypocotyl without any

change. At the region of transition the four xylem groups continue their courses further down without change in position so that the root vascular structure retains a diagonal tetrarch condition. Consequently, transition takes place here by splitting and fusion of the phloem groups. The present study has, therefore, brought to light one more instance of the rare diagonal structure of the seedling.

Another interesting feature met with in the seedling of *Grevillia robusta* is the formation of a knob-like swelling at the base of the hypocotyl where transition takes place. Its function is presumably similar to that of the "peg" found in the Cucurbitaceæ. The formation of the knob-like swelling is, however, rather variable.

In conclusion, it may be stated that while characters of seedling structure are often regarded to be of phylogenetic value, there are numerous cases where correlations can be made only with extreme caution. In the case of the Leguminosæ, however, the very comprehensive work of Compton (1912) has revealed the fact that the root structure in the family is very constant, especially in the subfamilies Mimosoideæ and Cæsalpinioideæ, and also in the tribe Phaseoleæ belonging to the subfamily Papilionatæ. His work also shows that there are correlations between the mature plant habit and the seedling on the one hand, and between the latter and a particular type of root symmetry on the other. Bancroft (1930) remarks that while they may be so only in certain restricted circles, as the Leguminosæ, " no general rule can be laid down for the whole angiosperms ". However, it would be of interest to find out if any uniformity in seedling structure prevails in the Proteaceæ. This can be done only after a number of representative genera are studied, and the results of such studies will probably also throw some light on the systematic position of the family.

SUMMARY

The paper deals with the vascular anatomy of the seedling in *Grevillea robusta* Cunn.

The time required for germination is a long one, as much as a fortnight.

In some seedlings the base of the hypocotyl shows a knob-like swelling, while in others it is either very much reduced or even absent. The swelling is a localized cortical growth formed in accordance with the nature and direction of stimuli which initiate it. Its maximum development is seen when the seeds are germinated in a flat position. It is helpful in the opening of the seed-coat during germination in the same way as the " peg " formed in the Cucurbitaceæ.

The root shows in transverse sections a diagonal tetrarch vascular cylinder with a large amount of pith. The pericycle has about two layers of cells, and the endodermis can be clearly seen with the Casparian thickenings formed along the radial walls of the cells.

The apex of the root has a common meristem which appears in the form of a shallow bowl in longitudinal sections. This meristem

gives rise to the cortical and stelar tissues to the inside and to a well-developed root-cap towards the outside.

The secondary root arises opposite one of the protoxylem points of the primary root. Some of the cells of the pericycle and endodermis in the primary root divide in tangential and radial planes to give rise to the secondary root. The cells formed by the pericycle give rise to the cortical and stelar tissues, while those formed by the endodermis develop into a " temporary " root-cap for the secondary root. Very soon an apical meristem is formed in the secondary root and further growth takes place by the activity of this meristem.

The transition region is located at the base of the hypocotyl where the knob-like swelling is seen. During transition, the phloem groups divide and the halves belonging to adjacent groups reunite in pairs outside the xylem. In the xylem groups there is only a re-orientation of the protoxylem and metaxylem elements during transition.

The hypocotyl shows four collateral endarch bundles in transverse sections. Very near the cotyledonary node these bundles show cambiform cells, thereby indicating that secondary activity commences very early here.

Anastomoses are formed between the hypocotyl strands at the cotyledonary node, after which the main vascular supplies of the two cotyledons are formed. There are three main vascular strands in each cotyledon, a midrib strand and two marginal strands. The latter form branches to supply the basal lobes of the cotyledon, namely the *auricles*.

The primary vascular structures of the epicotyledonary shoot are not at first directly connected to the hypocotyl strands, but become so only later after secondary activity commences at this region. The growth of the epicotyledonary shoot is rapid and its leaves are borne according to a two-fifths spiral phyllotaxy.

In conclusion, it is my pleasant duty to thank Dr. P. Maheshwari, Dacca University, for kindly reading through this paper, and Professor M. A. Sampathkumaran, Head of the Department of Botany, University of Mysore, for the many courtesies and kind encouragement during the course of this work.

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FLORAL ANATOMY OF *NYCTANTHES ARBOR-TRISTIS* L.

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VERY little is known at present about the floral morphology of members of the Oleaceæ. There is some record of embryological observations on the family, but little is known about the vascular anatomy of the flower. There are only two earlier papers which say anything about the floral anatomy of this group. Eames (1931) in his paper on the theory of carpel polymorphism deals casually with the vascular supply of the gynœcium of *Syringa* and *Forsythia*. King (1938) in his study of the morphology of *Olea europea* has briefly described the anatomy of the flower and fruit. A preliminary investigation of some members at Benares showed that there are many interesting features to be discovered in this field (Joshi and Fotidar, 1940). This is the reason for continuing these investigations. A previous paper by the writer in this journal (Fotidar, 1939) dealt with the course of primary vascular bundles in the vegetative stem of *Nyctanthes arbor-tristis* Linn. The present contribution deals with the floral anatomy of the same species.

The small trees of *Nyctanthes arbor-tristis*, which are common throughout this country, bloom from August to October. The fragrant flowers are nearly sessile and borne in small, pedunculate, bracteate heads, forming terminal trichotomous cymes. They open during the night and fall to the ground by early morning. The calyx has an ovoid-cylindric shape and a nearly truncate margin. Corolla is salver-shaped, with a cylindric orange-coloured tube and 5-8 white obcordate spreading imbricate lobes. The two stamens are attached close to the top of the corolla-tube and are nearly sessile. The ovary is bilocular, with one erect anatropous ovule in each loculus, arising from near the base. It is topped by a cylindric style, ending in a shortly bifid stigma. The fruit is an orbicular, dorsally much compressed structure, which splits when ripe into two one-seeded parts.

Material for the investigation was collected at Benares from one of the trees growing in the Hindu University grounds. It was fixed, embedded in paraffin and microtomed according to the customary methods. Several combinations of stains were tried, but a combination of Safranin and Delafield's Hæmatoxylin was found most satisfactory. Flower buds alone were employed in the investigation except for the vascular anatomy of the ovule. Some seeds were also studied for the latter purpose. The particular flower

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sketched in Figs. 1–19 to illustrate the vascular supply possessed six petals.

STRUCTURE OF THE FLORAL PEDUNCLE AND PEDICEL

The vegetative stem of *Nyctanthes arbor-tristis* is distinguished by the presence of four inversely orientated cortical bundles in addition
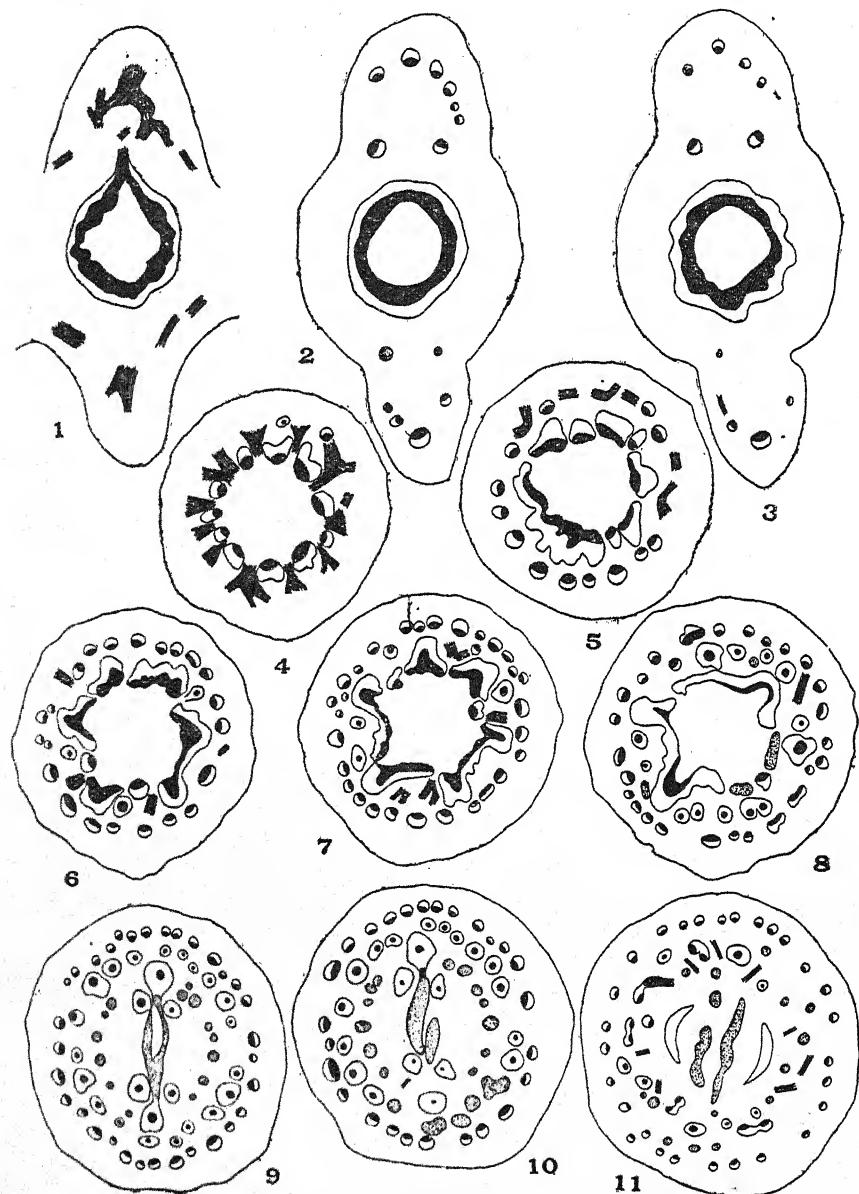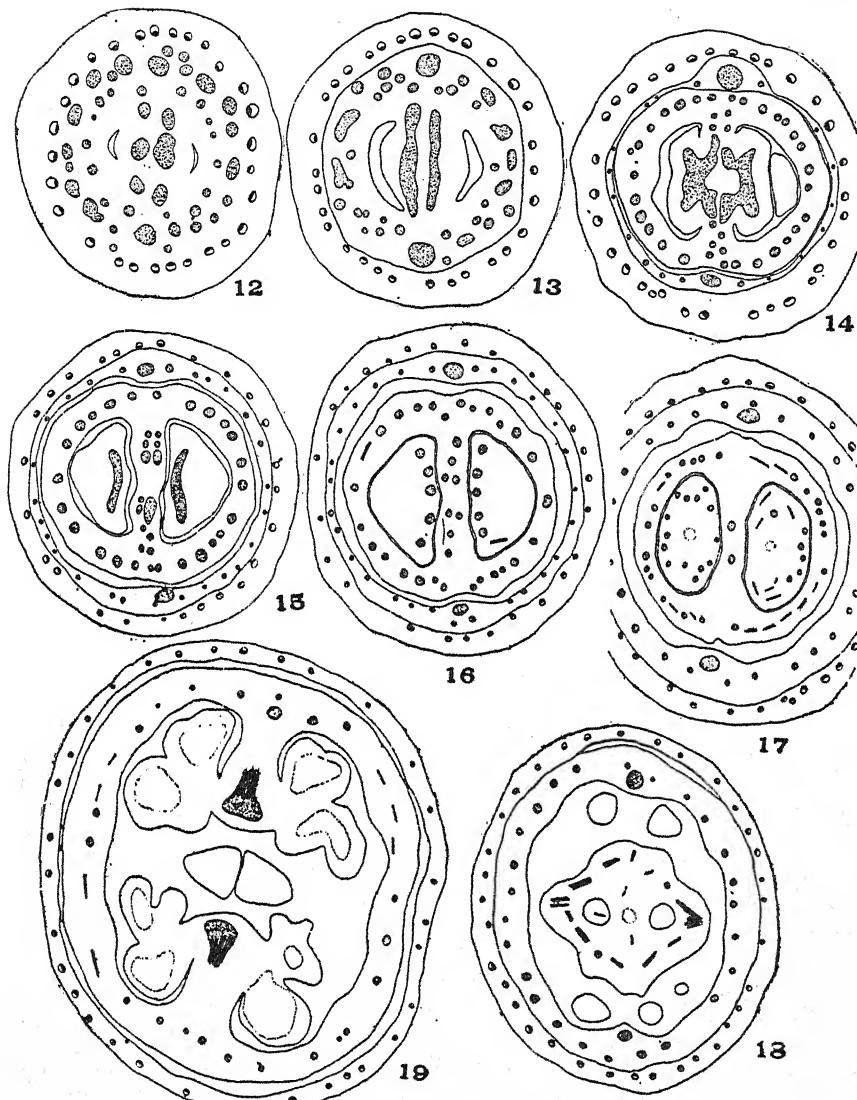
Figs. 1–19. *Nyctanthes arbor-tristis*. A series of transverse sections of a flower bud from the base of the pedicel (from the level of the last bracts on a peduncle) upwards to the level of the stigma and the base of the stamens. In bundles which are fully differentiated, xylem is shown black, phloem white. Undifferentiated vascular strands are shown by dots. For further explanation see text.   × 30.

to the normal ring, one cortical bundle being placed in each corner of the 4-angular stem (Fotidar, 1939). The same structure is seen in the floral peduncles. The vascular supply of the bracts, however,

is slightly different from that of the foliage leaves. The vascular supply of a vegetative leaf consists of a large arc-shaped central bundle, with a small bundle on either side. The bracts possess at their base and midrib an arc of 3–5 bundles of nearly equal size. Otherwise the origin of bract traces is very similar to that of foliage leaves. Figs. 1–3 illustrate the departure of vascular traces from a peduncle to the last pair of bracts. Above this level the very short pedicel of the almost sessile flowers is seen. One of the bracts in Fig. 1, the upper one, shows in its axil the departure of a vascular trace for an axillary flower, which has not developed. The pedicel, after the traces to the last pair of bracts have been given off, shows a structure, which again closely resembles that of the vegetative stem. It shows a central ring of bundles and four inversely orientated cortical bundles (Figs. 2 & 3). The only noteworthy feature is that very commonly the four cortical bundles are not equally developed on the two sides. Very often bundles on the side away from the parent axis are better developed than on the other. Thus the cortical bundles on the upper side in Figs. 2 and 3 are large, well differentiated into xylem and phloem, show their orientation clearly and continue into the thalamus up to the level of the sepal traces, while those of the lower side are small, undifferentiated into xylem and phloem and soon fade out in their course towards the thalamus. One of them has already disappeared in Fig. 3, even before the separation of the bracts from the floral axis.

Vascular Supply of the Calyx

The vascular supply of the calyx and corolla in *Nyctanthes* shows much irregularity and the number of traces coming out from the thalamus stele and supplying these parts is not quite definite. The vascular supply of the calyx in the flower sketched in the above illustrations consists of eleven main traces (Fig. 4). These vary a great deal in their size, some being much larger than the others. Each leaves a separate gap in the axial stele, breaking it into a ring of eleven collateral bundles of unequal size. Some of these traces remain undivided. Others, as can be seen from Fig. 4, divide immediately after their origin from the stele into two or three branches. The cortical bundles of the pedicel, which run into the thalamus, now merge into the calyx traces. Two of them are seen in Fig. 4 lying close to two calyx traces. At the level of Fig. 5, they have united with the latter and disappeared. All the calyx traces divide into 30–40 bundles before the separation of the calyx from the thalamus (Figs. 5–11), and these run right up to the end of the truncate calyx (Figs. 12–19). The calyx traces and their branches have a collateral structure throughout their length.

Vascular Supply of the Corolla

After the departure of the calyx supply, the gaps left in the stele of the receptacle by the smaller calyx traces close up, but those left by the bigger ones remain open. Thus the receptacular stele now does not form a complete ring, but is broken into four or five

separate bundles (Figs. 5–8). These are quite large and roughly arc-shaped. Six traces are now given off, corresponding to the number of the corolla-lobes in the flower, each leaving a separate gap in the vascular ring (Figs. 5–8). These traces show a concentric structure, consisting of a central strand of xylem surrounded by phloem on all sides. Further, like the calyx traces, they do not all behave in the same manner. Some of them pass outwards as they are. Others divide into two, three or four bundles immediately after their origin. The vascular ring of the thalamus after the departure of this second whorl of traces,—they are not merely corolla traces,—becomes weak and consists mainly of undifferentiated procambial elements. The six concentric traces divide to form approximately twenty bundles, arranged in a ring within the circle of calyx bundles (Fig. 9). These bundles at the level at which the stamen traces are given off divide rather in an irregular fashion, some transversely, others longitudinally (Figs. 10–13). This results in the formation of two rings of bundles. The outer one supplies the petals, while the inner one forms a part of the carpellary vascular system (Fig. 14). As soon as this division between the corolla traces and the carpellary traces has occurred, these bundles lose their well differentiated concentric structure. They are now weakly developed and consist only of undifferentiated elements. Those going to the corolla are particularly poorly developed. As they pass through the corolla-tube, which is very thin and closely appressed to the calyx (Figs. 15–19), the weak corolla bundles divide still further. Thus a very large number of very fine vascular strands is formed, and ultimately each corolla lobe receives about ten very small bundles.

Vascular Supply of the Stamens

The stamen traces arise from the receptacular stele after the departure of the dorsal carpellary traces. The origin of the latter is described later. After their departure the stele of the thalamus gives off two large traces, one on either side, opposite to each other (Figs. 9–11). These, like the conjoint corolla and carpel traces, have a concentric structure. After their origin they pass out into the corolla-tube, running through its greater length without any change, except that they lose much of their differentiation (Figs. 12–19). They pass out into the two sub-sessile epipetalous stamens about the level of the stigma, each stamen being supplied by one of them (Figs. 18 & 19).

Vascular Supply of the Gynœcium

When the corolla traces have departed, the vascular cylinder of the floral receptacle gives off a large number of small traces on two sides (Figs. 9 & 10). They are situated in a plane at right angles to that formed by the staminal traces and their number on either side varies from about six to eight. They are poorly differentiated and gradually merge into the bundles cut off towards the

inside by the corolla traces. Together these constitute the main vascular supply of the wall of the ovary or the dorsal vascular supply of the carpels.
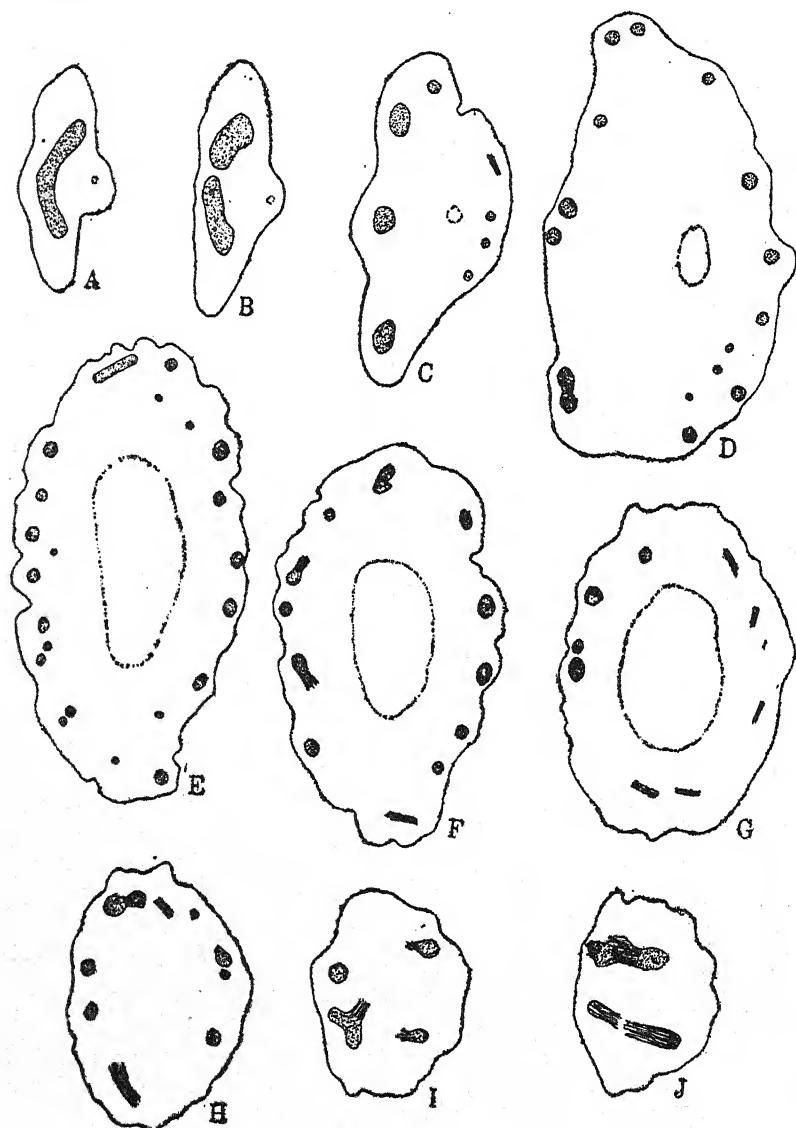
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Fig. 20, *a–j*. *Nyctanthes arbor-tristis*. A series of transverse sections of a seed from its base to the apex to show the extensive development of the integumentary vascular system. For further explanation see text. × 25.

At the level the two stamen traces are given off, the central vascular cylinder, which is now transversely much compressed, gives off laterally two pairs of concentric vascular bundles (Figs. 8 & 9). These run for some distance very close to the staminal traces. The remnants of the receptacular stele continue upwards as strands of undifferentiated vascular tissue. Higher up these break into about five pairs of vascular bundles (Fig. 12). The two pairs of concentric traces may give off a few branches which pass into the dorsal wall of the carpels, but ultimately they merge into the five pairs of ventral bundles and all of them unite together to form two broad vascular strands (Figs. 13 & 14). These broad strands give off a few branches from their ends, which run into the ovary wall, but they mainly supply the ovules. Each sends out a large trace for the ovule on its side (Figs. 14 & 15). After the departure of the ovular supply, the broad ventral strands again break up into smaller bundles, the large majority of which pass out to the dorsal side of the carpels. Only one or two of them remain in the ventral position (Figs. 16 & 17). No further changes occur till the base of the style is reached. Just below it, the various vascular bundles in the ovary wall begin to move towards one another and unite (Fig. 18). Ultimately, one or two only or none continue into the style. These also fade away as we reach the stigma (Fig. 19).

Vascular Supply of the Ovule

The vascular supply of the ovule in this plant is complicated by the prolific development of integumentary vascular system. Therefore, it was thought worth while to trace it in a detailed manner and it is fully illustrated in Fig. 20. To begin with each ovule receives a single crescent-shaped vascular trace (Fig. 20 *a*). This soon forks into two (Fig. 20 *b*). These branches then divide, and some of the branches pass out towards the other side of the ovule (Fig. 20 *c*, *d*). At this level the ovule is more or less compressed and has a number of ridges and furrows on its surface. These ridges correspond to the vascular strands inside. The single integument is quite massive. The vascular bundles ramify copiously through this integument. A mature seed about its middle shows approximately 25–30 such bundles (Fig. 20 *e*). Higher up these bundles begin to approach one another and fuse in an irregular manner (Fig. 20 *f*, *h*). Some of the smaller ones fade away. Only the larger ones continue into the apex of the ovule and gradually these also unite to form two strands (Fig. 20 *i* & *j*). Finally, these also disappear.

Summary

The floral peduncle and the short pedicel of *Nyctanthes arbortristis* agree with the vegetative stem in possessing four inversely orientated cortical vascular bundles in addition to the normal ring. The number and behaviour of the calyx traces is not very definite. There are about eleven original traces of unequal size and these divide irregularly to form about 30–40 bundles. The number of

corolla traces is the same as the number of corolla-lobes. These are conjoint at their base with some of the dorsal carpellary traces. The corolla, stamen and some of the carpellary traces are concentric. The vascular supply of the carpels consists of many bundles, about 15, which arise in a complex order. The ovule is characterised by the development of a strong integumentary vascular system by the division of the single ovular trace.

This investigation was carried out at the Benares Hindu University and the author takes this opportunity to express his cordial thanks to Dr. A. C. Joshi, D.SC., F.N.I., for suggesting the problem and help in the preparation of this paper.

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VARIATION IN THE PHOTOSYNTHETIC RATE IN *ELODEA*

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This work was started as early as in 1928 when an apparatus was constructed to record the rate and volume of gas evolved during photosynthesis in *Elodea*. The construction and working of the apparatus formed the subject-matter of the paper published by the author in 1929. While testing the working of the apparatus several records were taken and these showed some fluctuations in time-interval between the bubbles although the conditions were maintained constant. Subsequently this aspect was tackled again and the work extended over several months during which period thousands of readings were taken. In all these investigations the bubble counting method was resorted to, since ' the rate at which these bubbles are liberated may be taken as a measure of the relative rate of photosynthesis ' (Spoehr, 1926).

There are many contributions to our knowledge of the factors and their influence on photosynthesis. The outstanding contribution is by Blackman (1905) who has discussed elaborately the interaction of the several factors and its effect and the importance of the limiting factor in the process of assimilation. The reviewing of the various aspects of photosynthesis by Walter Stiles (1925), Spoehr (1926) and Barton Wright (1930) makes this point clearer.

The factor of fatigue was introduced by Pantanelli (1903), although the exact way in which this is likely to affect photosynthesis is not explained by him. Ursprung (1917) first introduced a new factor, ' Solarisation ', and this is described as inactivation of photosynthesis of a plant by continued exposure to light. Yap (1920) and Stanescu (1924) have done further work on ' Solarisation ' enabling one to conclude that the fatigue factor as understood by Pantanelli is actually the result of solarisation. Miller (1931) also draws the same conclusion. Although this is an important factor, still its effect results in a temporarily pathological condition on account of the partial destruction of the protoplasm, or decomposition of the protoplasm or chlorophyll or both.

The present paper is an attempt of the author to show that the periodical depression is independent of solarisation and that this is of very great significance in photosynthesis on account of its frequent occurrence and appreciable influence on the rate of assimilation. The way in which this affects the photosynthetic rate is explained in this paper.

4

METHOD

Elodea was the plant selected for work. A special type of bubbler (Wilmott, 1921) was constructed and used throughout the investigation. The water in which the plant was growing was used, and 1% solution of sodium bicarbonate was prepared with this water. Arrangements were made to ensure a steady supply of the solution and slow draining of the old solution with minimum disturbance to the twig. Necessary precautions were taken to maintain the conditions constant throughout the experiment. The twig was exposed to diffuse light and direct sunlight and readings were taken. Short time records of 20–30 minutes' duration were taken and the time-interval between successive bubbles was noted down. Fresh twigs were selected after one or two records. A few of the records have been presented below in the form of graphs.

OBSERVATIONS

Three graphs have been introduced in this paper to show the time-variation in the rate of bubbling. Graphs I and II are from records taken during a bright day with the apparatus near the

Graph I

Graph II
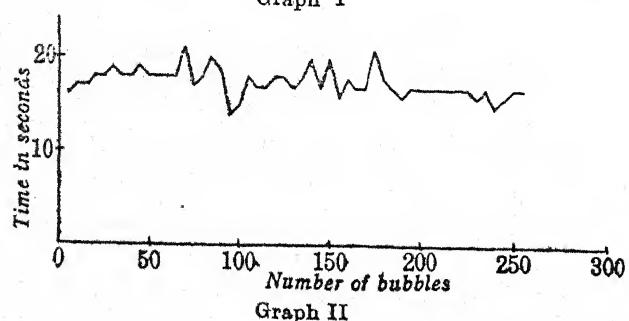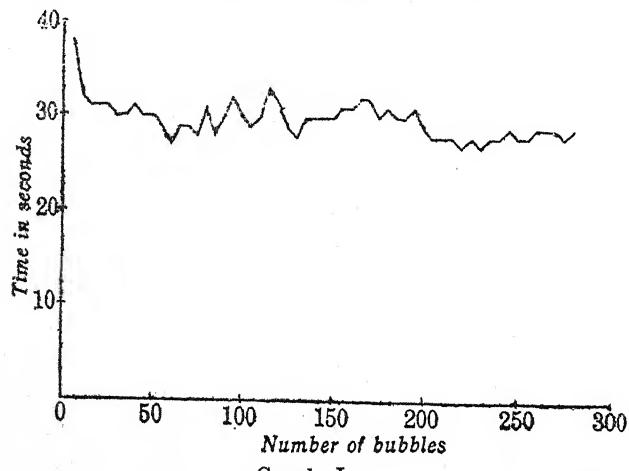
window and with the readings at five bubble intervals. This has certain advantages over the one bubble interval records inasmuch as the errors in the fractions of seconds have been eliminated thus making the records more accurate. Certain interesting features are noticed in these two records. The period of activity is invariably followed by a period of reduced rate, the former showing pronounced fluctuations while the latter is almost uniform. During a period of activity the fluctuations in the rate are highly pronounced, the shortest and the longest time taken for 5 bubbles (Graph I) being 27 and 33 seconds respectively. The two graphs also indicate that the twig is able to adjust itself to a higher rate of assimilation after a series of attempts these invariably alternating with reduced rates as indicated by the hollows and peaks in the graphs. Even during a period of almost uniform rate a few fluctuations are noticed but these are neither of appreciable magnitude nor of regular occurrence.

Graph III is from readings taken between 10·15–10·45 A.M., with the sun falling directly on the specimen. The water in the trough was allowed to absorb the heat of the sun. Thus at the end of the experiment the temperature had risen by nearly 1° C. While all other factors were constant only temperature happened to be the variable factor. The graph can be divided into five regions, *a, b, c,*
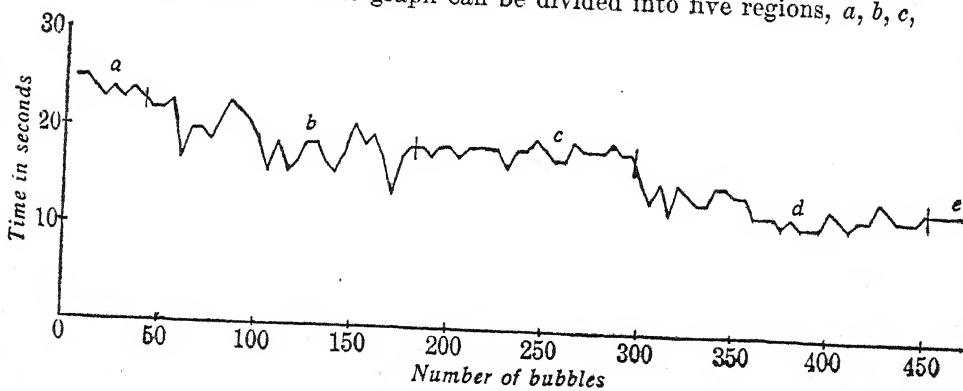
Graph III

d, e, for the sake of explanation. The regions *a, c* and *e* indicate periods of almost uniform activity, while *b* and *d* are periods of intense activity with significant fluctuations in the rate from time to time. The average time of five bubble interval during these five periods are nearly 24, 19, 19, 14 and 14 seconds respectively; and the time for *b, c* and *d* happens to be 530, 435 and 427 seconds respectively, indicating thereby that at a higher rate the period of activity is of shorter duration. During a period of active assimilation most pronounced fluctuations in the rate are met with. This is seen in the regions of *b* and *d* but more so in the region of *b* where the difference between the maximum and minimum rates happens to be 9 seconds, the two rates being 14 and 23 seconds respectively for five bubble interval. At the commencement this difference is greater

but later on this becomes smaller, this being illustrated by the two regions *b* and *d*. Although there is not any difference between the average rates of *b* and *c* or of *d* and *e* the fluctuations are however conspicuous in *b* and *d*. Thus the graph indicates a possible effort of the plant to adjust itself to a higher rate by a series of attempts.

Conclusion

The above-mentioned observations indicate that photosynthesis is not a uniform process even under constant conditions. There are periods of active assimilation alternating with stages of reduced rate and showing marked variation during vigorous assimilation. The period of reduced rate indicates a stage of probable fatigue. It is also noticed that the twig adjusts itself to a vigorous rate of assimilation by a series of attempts as indicated by the conspicuous peaks and hollows in the graphs. Regarding the number of these attempts it may be stated that there will be five or six, the magnitude of these depending on the rate of photosynthesis and the condition of the twig. The period of active assimilation may be taken to be 7 to 11 minutes depending on the rate, and this is invariably longer than the period of depression.

Summary

1. *Elodea* is the plant selected for the present investigation.

2. Depression in the rate of photosynthesis seems to be an important feature manifesting itself either momentarily or periodically.

3. This makes its appearance whether the plant assimilates in diffuse light or in bright sunlight.

4. There are periods of vigorous assimilation alternating with those of reduced activity, the latter indicating a stage of probable fatigue.

5. The period of activity will be between 7–11 minutes depending on the rate of photosynthesis and this will be invariably longer than the period of depression.

6. During a period of active assimilation there are invariably 5–6 fluctuations, these being highly pronounced when the twig is adjusting itself to a higher rate of assimilation.

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MORPHOLOGICAL AND CYTOLOGICAL STUDY OF THE RUST ON *HEDYOTIS STYLOSA*

[*Chrysocelis ascotela* (Syd.) Comb. nov.]

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(Communicated by M. A. Sampathkumaran)

In 1940 the writer collected at Kodaikanal a rust on *Hedyotis stylosa* Brown, which appeared to fit admirably *Blastospora ascotela* Syd. This latter rust is also on the same host and was founded by Sydow (Sydow and Mitter, 1936) on material collected at Ootacamund by J. H. Mitter. Only sub-epidermal pycnia and telia have so far been reported and the rust is apparently a micro-cyclic species. Its teliospores are one-celled, hyaline, germinate immediately and are *reported* to be pedicellate.

The genus *Blastospora* was founded by Dietel (1908) to accommodate a rust on *Smilax Sieboldii*. It is characterised by superstomal telia which are formed externally on the leaf surface. The wall of the promycelium is not the continuation of the teliospore wall, and the promycelium is separated from the germinated teliospore by a septum, a feature peculiar to the genus. Mains (1938) who studied the type material of *Blastospora ascotela* did not find any of these distinguishing characters in the fungus, and he thought that the proper place for the rust is in the genus *Maravalia* to which he transferred it. This seemed to be the proper genus for the time being for the reception of the rust. In *Maravalia* the teliospores arise singly from compact hymenium ; they are pedicellate and this latter fact separates the genus from *Chaconia, Bitzea, Chrysocelis* and other genera. In placing the rust in *Maravalia*, Mains (1938) states that the telia are hypophyllous, densely grouped about the pycnia and that the teliospores are cylindric with hyaline, thin-walled pedicels.

A detailed morphological and cytological examination of the rust by the writer has revealed however several interesting features. It is true that pycnia (Fig. 7) are sub-epidermal, but they are also amphigenous with ostiole and paraphyses. Telia are also sub-epidermal and mostly hypophyllous, but amphigeneous ones have also been noted. The telial initials are formed by the concentration of uni-nucleate hyphæ in the intercellular spaces, forming plectenchyma in the sub-hypodermal position. Before initiation of the dicaryon phase cell fusion takes place. There is no definite grouping of these fusion cells but any two adjacent cells might fuse. The

nucleus migrates through a small pore formed by the dissolution of the septa. The binucleate cell thus formed elongates lengthwise and by cell divisions forms two to three cells which are superposed (Fig. 9). The topmost cell elongates and forms the teliospore. The spores become cylindric and are binucleate (Fig. 2). At a later stage the nuclei fuse giving rise to a syncaryon (Fig. 4). The basal cell remains as such without any change and measures about 4–5×7–$12\ \mu$. In a mature telium all the teliospores are sessile and soon after maturity germinate within the sorus. The spores are clavate, and thin-walled without any germ pore. Promycelium is formed by the prolongation of the spore apex (Fig. 6). The tip of the promycelium is thickened and this helps in breaking through the epidermal layer (Fig. 3).

Following germination, the outlines of the teliospore and the promycelium cannot be differentiated as it is uniformly broad throughout. The nucleus and the cytoplasm migrate towards the apical region of the promycelium, and a cell is cut off at the level of the epidermis (Fig. 8). By successive divisions a four-celled promycelium is formed, and in most of the cases observed the basidium becomes recurved. Sterigmata are formed which abstricts off uninucleate basidiospores above the level of the epidermis (Fig. 5).

Following germination of the teliospore the basal cell becomes active. Before the prolongation of the spore apex to form the promycelium, the basal cell measures 4–5×7–$12\ \mu$. It soon begins to elongate as indicated by measurements taken at various stages of germination of the teliospore. After the formation of promycelium the basal cell elongated up to 17–$21\ \mu$. After the abstriction of the basidiospores the promycelium collapses and the cell walls become gelatinous. But the basal cell remains persistent with two nuclei and cytoplasm. It remains as an attenuated fusiform structure, measuring 65×30–$40\ \mu$ in length. In some ten cases it was found to measure about 40–$70\ \mu$. This is in accordance with the measurements given by Sydow and Mitter (1936), where it is recorded as 40–$70\ \mu$.

It is manifest that the structure mistaken for a pedicel by Mains (1938) in *Maravalia ascotela* is the elongated basal cell. No doubt, the pedicels in the stipitate rusts are formed by the elongation of the stalk cell. But we have to take into consideration the mature spore before germination for determining the stipitate or sessile character of the teliospore. In the rust on *Hedyotis stylosa* it is definitely sessile even up to the early stages of germination. If the basal cells are considered to be pedicels, they would not elongate indefinitely as they do in the rust under study and as indicated by measurements.

Because of the sessile nature of the teliospores the identity of the rust requires to be reconsidered. Sub-epidermal telia with cylindric thin-walled hyaline teliospores germinating immediately have been recorded in *Bitzea* and *Chrysocelis*. In the genus *Bitzea*, erected by
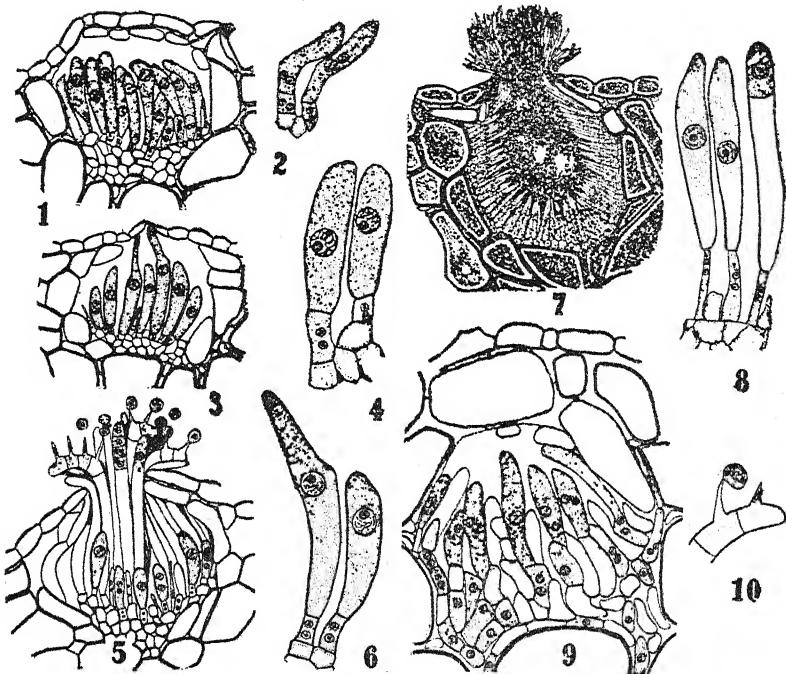
Text-figs. 1–10.—Fig. 1. Section through a mature telium with sessile, clavate teliospores (× 400). Fig. 2. Young teliospores showing two nuclei (× 400). Fig. 3. Showing the germinating teliospores breaking through the epidermis (× 400). Fig. 4. Mature teliospore (× 800). Fig. 5. Section through a telium with germinating teliospores (× 400). Fig. 6. Early stage of teliospore germination showing the formation of promycelium by the prolongation of spore apex (× 800). Fig. 7. Camera lucida drawing of a pycnium (× 400). Fig. 8. Showing the elongation of the basal cell after teliospore germination (× 800). Fig. 9. Section through a young telium (× 800). Fig. 10. Development of sporidium (× 800).

Mains (1939), the pycnia are sub-cuticular, whereas in *Chrysocelis* they are sub-epidermal. The rust on *Hedyotis stylosa* has sessile teliospores with sub-epidermal pycnia, and these characters justify its inclusion in the genus *Chrysocelis*.

The genus *Chrysocelis* was founded by Lagerheim and Dietel (1913) to include two species of rusts *C. Lupini* Lagerh. and Diet., on *Lupinus* sp., and *C. Muehlenbackiæ* Lagerh. and Diet., on *Muehlen-beckia*. In *C. Muehlenbeckiæ* only pycnia and telia have been recorded. In *C. Globbæ* Syd. recorded by Sydow and Petrak (1931) the telia are hypophyllous, and the sori are covered by epidermis which is ruptured by the germinating teliospores. The spores are uni-cellular, clavate and sessile germinating immediately. The promycelia are quickly perishable. The rust on *Hedyotis stylosa* resembles these forms in a general way. The writer proposes to transfer this rust to this genus and make a new combination *Chrysocelis ascotela* (Syd.).

The elongating basal cell is not without significance. Its mode of development suggests that it helps in pushing out the promycelia above the epidermal layer, and the basidiospores are formed exteriorly on the leaf surface. One might assume that pedicellate teliospores in forms with sub-epidermal, non-erumpent telia have better chances of having their basidia extruding out of the host tissue, than forms with sessile teliospores. In the latter forms the promycelia have to develop to a greater length. The elongated basal cell of *Chrysocelis ascotela* only simulates a pedicel, and in this respect it might be an intermediate form connecting the two genera *Maravalia* and *Chrysocelis*.

DESCRIPTION OF THE RUST

Chrysocelis ascotela (Syd.) comb. nov.

Pycnia amphigeneous, distributed in concentric rings in yellow patches on the leaf, sub-epidermal, flask-shaped, with ostiole and paraphyses. Telia amphigeneous, mostly hypophyllous, in groups around pycnia, sub-epidermal, covered with epidermis in early stages, soon naked following germination, covered with a whitish pulverulent mass; teliospores clavate, sessile, hyaline and thin-walled, measuring 44–61 × 9–14 μ, germinating immediately by the prolongation of the spore apex producing the promycelium, basidia exserted above the epidermis; basidiospores thin-walled, spherical measuring 12 × 11 μ. Basal cell 4–5 × 7–12 μ. in mature spores, elongating after germination up to 40–65 μ.

Hab.—On leaves of *Hedyotis stylosa* Brown, leg., Thirumalachar, Kodaikanal Hills, South India, 18-10-1940.

SUMMARY

1. The rust on *Hedyoits stylosa* Brown is a microcylic form with sub-epidermal pycnia and telia.

2. The teliospores are clavate, sessile, thin-walled and germinate immediately after maturity. These characters indicate that the rust is a species of *Chrysocelis* and a new combination *Chrysocelis ascotela* (Syd.) is proposed.

4. The basal cell elongates after the germination of the teliospore and this helps the promycelium to be extruded out of the host tissue.

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ALTERNARIA ON LEAVES OF SUNFLOWER IN INDIA

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(Communicated by S. R. Bose)

INTRODUCTION

In recent years several species of *Alternaria* have been reported in India as causing diseases of the following plants—*Vicia faba* L., *Citrus sinensis* Osbeck, *Nicotiana tabacum* L., *Gossypium* sp., *Capsicum frutescens* (Dutt, 1937). Dey (1933) records *Alternaria lini* Dey on the inflorescence of *Linum usitatissimum* L., Uppal *et al.* (1938), *A. burnsii* Uppal, Patel and Kamat on fruits of *Cuminum cyminum* L. Young (1929) lists nearly 80 species of *Alternaria* attacking various plants; he mentions only two members of the Compositæ family (*viz.*, soncus and sunflower) to be infected with *Alternaria*. *Alternaria tenuis* on leaves of sunflower has been recorded both from Europe (Oudemans, 1923) and America. *Alternaria tenuis* Nees is reported in this paper as occurring on the leaves of sunflower (*Helianthus annuus* L.) for the first time from India. Mohendra (Butler and Bisby, 1931) has already noted the occurrence of the same species on leaves of *Saccharum officinarum*.

Incomplete descriptions, mutations, secondary development of spores, dwarfing of spores in culture, and facultative parasitism resulting in large host ranges, all these have caused great difficulty in the classification of species of *Alternaria* and *Macrosporium*. Elliott's paper (1917) has been of considerable help in clearing away the confusion in the delimitation of both these genera. *Macrosporium* and *Alternaria* by reason of their muriform spores are placed in the section *Dictyosporæ* of the family *Dematiaceæ* of the order *Moniliales*; these muriform spores separate them from the genera *Cladosporium* and *Helminthosporium*, which in some species are similar in respect of many characters. The separation of the genera *Alternaria* and *Macrosporium* rests solely on the catenulation of the spores in the former genus. The most characteristic feature about the spores of these fungi is their shape which is an immediate index to the genera, and generally to the species. According to Elliott, all obclavate, ovate, cuneate, pointed or beaked spores belong to *Alternaria* and under suitable conditions form chains. There is variation in shape within a single species, but in most cases under natural conditions the shape of the spore, combined with its size, helps to identify the species.

" Echinulation of spores is not a constant character, hence this feature is generally regarded as having no value generically "

(Wiltshire, 1933). Beaks vary enormously in length and thickness. Moreover, the beak of the fungus on the natural host may be very different from that developed in culture, *e.g.*, *Alternaria gossypina* (Thüm). On the cotton plant Hopkins finds only a short beak, but in culture this fungus produces a long beak. *A. tenuis*, on the other hand, has a short beak on the leaves and practically no beak at all in culture ; only in old cultures a few spores with short beaks have been found. The length of the beak is usually fully developed, but it may also vary considerably in cultures of a single species, and occasionally spores have been observed with no beak at all even in the long-beaked species.

Elliott tentatively divided the genus *Alternaria* into groups of species having similar spores. The following groups were suggested by him :—

1. The *A. tenuis* group.—This group is characterized by spores ranging from $11-50 \times 7-20 \mu$. Spores are generally broad and muriform.

2. The *A. brassicæ* group.—This group contains spores ranging from $35-120 \times 10-30 \mu$. The spores have often long beaks.

3. The *A. herculeæ* group.—To this group belong spores similar in form but much larger than those of the *A. brassicæ*.

4. The *A. cucumerina* group.—This group is similar in spore-form to that of *A. brassicæ* group but the spores are uniformly wider, more muriform, and generally shorter.

5. The *A. sonchi* group.—Spores are large-celled with a distinct obtuse apex. The range in size is $50-125 \times 12-25 \mu$.

6. The *A. brassicæ* var. *microspora* group.—The spores of this differ from those of *A. tenuis* in being uniformly narrower and less muriform, longitudinal septa being seldom formed.

A more extended study of the genus might lead to an increase in the number of groups proposed.

SYMPTOMS

In June 1940, in the Garden attached to the Botany Department of the Carmichael Medical College several leaves of sunflower plant were found to have turned brown completely. The age of these plants was nearly three months. Many of them showed numerous small spots on the upper surface of the leaves. Some leaves were studded all over with these spots (Fig. 1). The spots were small and oval in shape. The spotted areas were frequently depressed and whitish in colour. In cases of vigorous attack, these spots may be numerous and may extend along the whole leaf surface owing to the coalescence of adjacent spots. In several instances in later stages of the disease the affected areas were perforated irregularly, due to the falling away of the dead tissue, resulting in what is known as " shot hole ". Generally, in mature plants, the upper leaves showed symptoms of the disease. Distinct conidia of *Alternaria tenuis* Nees were detected on leaves examined. The conidia

are ovate with a short beak and a slender base. They are muriform, singly borne, and light-brown in colour.

THE PATHOGEN

Small bits of infected leaves were thoroughly washed in water and transferred to one per cent. aqueous solution of silver nitrate. After one minute these were removed and dropped in a sterilised one per cent. solution of sodium chloride to precipitate the disinfectant and were readily transferred to malt-agar culture tubes. From most of the pieces *Alternaria* appeared in the course of seven to ten days. The fungus isolated was identified as *Alternaria tenuis* Nees.

The fungus was grown on the following media :—

Brown's synthetic medium (Glucose 2 gm., Asparagin 2 gm., K_3PO_4 1·25 gm., $MgSO_4$. $7H_2O$, 0·75 gm., Agar 20 gm., and water 1000 c.c.) ; 3% malt-agar medium.
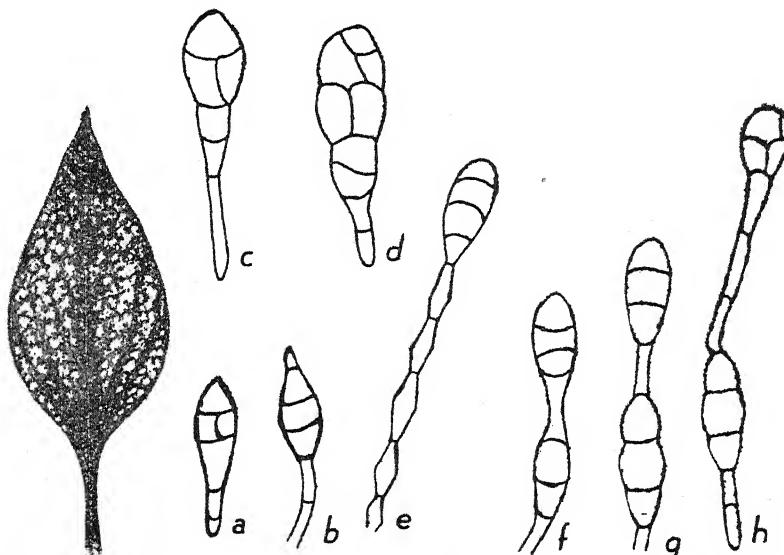
Fig. 1.   Fig. 2.

Fig. 1.   A leaf of sunflower spotted due to *Alternaria tenuis.* (Reduced.)

Fig. 2.   Conidia of *Alternaria tenuis.* *a*, young conidium. *b*, a beaked conidium from old culture. *c* and *d*, two mature conidia. *e*, a conidium with conidiophore. *f* and *g*, conidia in chains of two. *h*, conidia in chain with elongated stalk. [Magnification × 692].

CHARACTER OF THE FUNGUS IN CULTURE

The mycelium is septate, hyaline when young, turning light-brown later. Conidiophores are simple, or sometimes branched, septate, straight or bent and light-brown in colour.

In culture the conidia are usually singly borne, sometimes in chains of two to four, but catenulation is more common in older cultures. They are ovate, sometimes clavate with a slender base and rounded apex (Fig. 2). The conidia are variable in form as well as in size, but are generally broad and muriform. The number of transverse walls varies from two to seven, but it is usually from three to four. Sometimes individual conidia develop a large number of longitudinal and oblique septa resulting in an irregularly shaped cell. In colour the conidia are dirty-brown. These germinate by germ-tubes which may arise from either the tip or the basal stalk, or from any of the side walls. These conidia easily get detached.

The growth of the fungus was studied on the media given below; the proportions of the constituent chemicals in each medium correspond to that used in preparing Brown's synthetic medium. Observations were recorded on the tenth and twelfth day after the subculture and are given in Table I.

TABLE I

Growth Features of the Fungus (Alternaria tenuis) *on Different Media*

Medium	Rate of growth	Colony colour	Conidia
Glucose + K_3PO_4 + $MgSO_4$ + Asparagin + Agar (Brown's synthetic medium)	Good growth	Greyish black colony, reverse turns black	Abundant normal muriform conidia, majority of the conidia are 3–4 septate, but the range of septation is 2–7; chain formation common
K_3PO_4 + $MgSO_4$ + Asparagin + Agar	Medium growth	Whitish colony	Conidia few, 2–4 septate
Glucose + $MgSO_4$ + Asparagin + Agar	Medium growth	Colony greyish black at centre, white along sides	Conidia many, Septa 2–4, chain formation occasional, longitudinal septa rare
Glucose + K_3PO_4 + $MgSO_4$ + Agar	Very slight growth	Very thin white colony	Conidia very few, hyaline
3 % malt agar	Good growth	Floccose dirty green colony, reverse turns black	Abundant normal muriform conidia, number of septation 3–5, chain formation common

From the above table it is found that *Alternaria tenuis* from sunflower leaves produces abundant normal conidia in 3% malt agar and Brown's synthetic media ; in other media growth is checked appreciably, and in the medium lacking in asparagin growth is negligible.

TABLE II

Size of Conidia on Different Media

Medium	Average	Range
Glucose + K_3PO_4 + $MgSO_4$ + Asparagin + Agar	$26 \times 7 \mu$	$20-44 \times 6-8 \mu$
K_3PO_4 + $MgSO_4$ + Asparagin + Agar	$25 \times 7 \mu$	$21-36 \times 6-9 \mu$
Glucose + $MgSO_4$ + Asparagin + Agar	$21 \times 6 \mu$	$15-33 \times 6-9 \mu$
Glucose + K_3PO_4 + $MgSO_4$ + Agar	$18 \times 6 \mu$	$15-24 \times 6-8 \mu$
3% malt agar	$38 \times 10 \mu$	$21-48 \times 9-12 \mu$

The spores were measured after ten days' growth at 21° C. The results are recorded above in Table II. Spores produced in 3% malt agar medium were the longest, the next, in order of size, were the spores produced in Brown's synthetic medium ; shortest spores were formed in the two media lacking asparagin and K_3PO_4 respectively.

INOCULATION-EXPERIMENT

Leaves of sunflower were inoculated with conidia of *Alternaria tenuis* grown on 3% malt-extract agar at 21° C. in three ways : (a) by wounding the leaves, (b) by directly placing inoculum containing conidia on the leaf surface, and (c) by the spore-suspension method.

The fungus infected wounded leaves in the course of four days, and unwounded leaves within ten days, producing brown discoloured areas. By the spore-suspension method spots appeared after fifteen days. Examination of these spots showed the presence of *Alternaria tenuis*.

4a

For the identification of this fungus the writer is indebted to Dr. G. W. Padwick, Imperial Mycologist, and to Dr. M. Mitra, of the Imperial Agricultural Research Institute, New Delhi.

SUMMARY

Alternaria tenuis Nees, causing a leaf-spot disease of sunflower, is reported for the first time from India. Pure cultures of the species have been studied. The conidia are ovate to clavate, with a slender base and rounded apex, and borne in chains of two to four. These range in size from $21–48 \times 9–12\ \mu$, and are muriform with 3–4 cross walls. The pathogenicity of the fungus has been proved by inoculation-experiment on living plants in the garden.

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STUDIES IN GESNERIACEÆ

Gametogenesis and Embryogeny of *Didymocarpus tomentosa* Wt.

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(Communicated by C. V. Krishna Iyengar)

SEVERAL genera of the Gesneriaceæ have been already studied by previous investigators. Hielscher, as early as in 1879, studied the anatomy and biology of *Streptocarpus*. The next contribution is by Balicka-Iwanowska (1889), who reported in *Klugia Notoniana* a binucleate chalazal haustorium and two uninucleate haustorial cells at the micropyle. In *Klugia zeylanica*, Schnarf (1917) finds that while there are two uninucleate micropylar haustoria, the chalazal haustorium has but a single nucleus. In both *Ramondia Nathaliæ* and *R. Serbica*, Glišic' (1924) observed that the chalazal haustorium is uninucleate as in *Klugia zeylanica*. Laurent (1923) records the presence of a binucleate chalazal haustorium in *Corytoloma cyclophyllum*. A similar condition is found in *Roettlera* sp., by Glišic' (1934), while in *Haberlea rhodopensis* the variations in the number of nuclei in the chalazal haustorium, and the occasional formation of a wall in the chalazal chamber indicate, according to the same author (Glišic', 1928), a condition less advanced than the others. Information regarding the exact sequence of divisions in endosperm formation in *Rhytidophyllum* (Cook, 1907) and *Streptocarpus* (Hielscher, 1879) is not available.

The present investigation deals with the development of the gametophytes and embryo in *Didymocarpus tomentosa* Wt. It records certain features not previously observed in the family. The material was collected in November 1939 at Himavadgopalaswamy Hill. Plants of *Didymocarpus tomentosa* were found growing in small crevices in the large boulders. The flowers and fruits in various stages were fixed in Bouin's fluid between 10–11 A.M. on the spot. A few plants were also grown at the Departmental Nursery at Mysore and the flowers and fruits of these were fixed in Navashin's fixative. Sections were cut at a thickness varying from 7 to 12 microns and were stained in Heidenhain's iron-alum hæmatoxylin.

POLLEN DEVELOPMENT

The primary archesporium is of hypodermal origin and cuts off a wall layer which, in turn, becomes three layered by division. While the outermost of these develops into the fibrous layer, the

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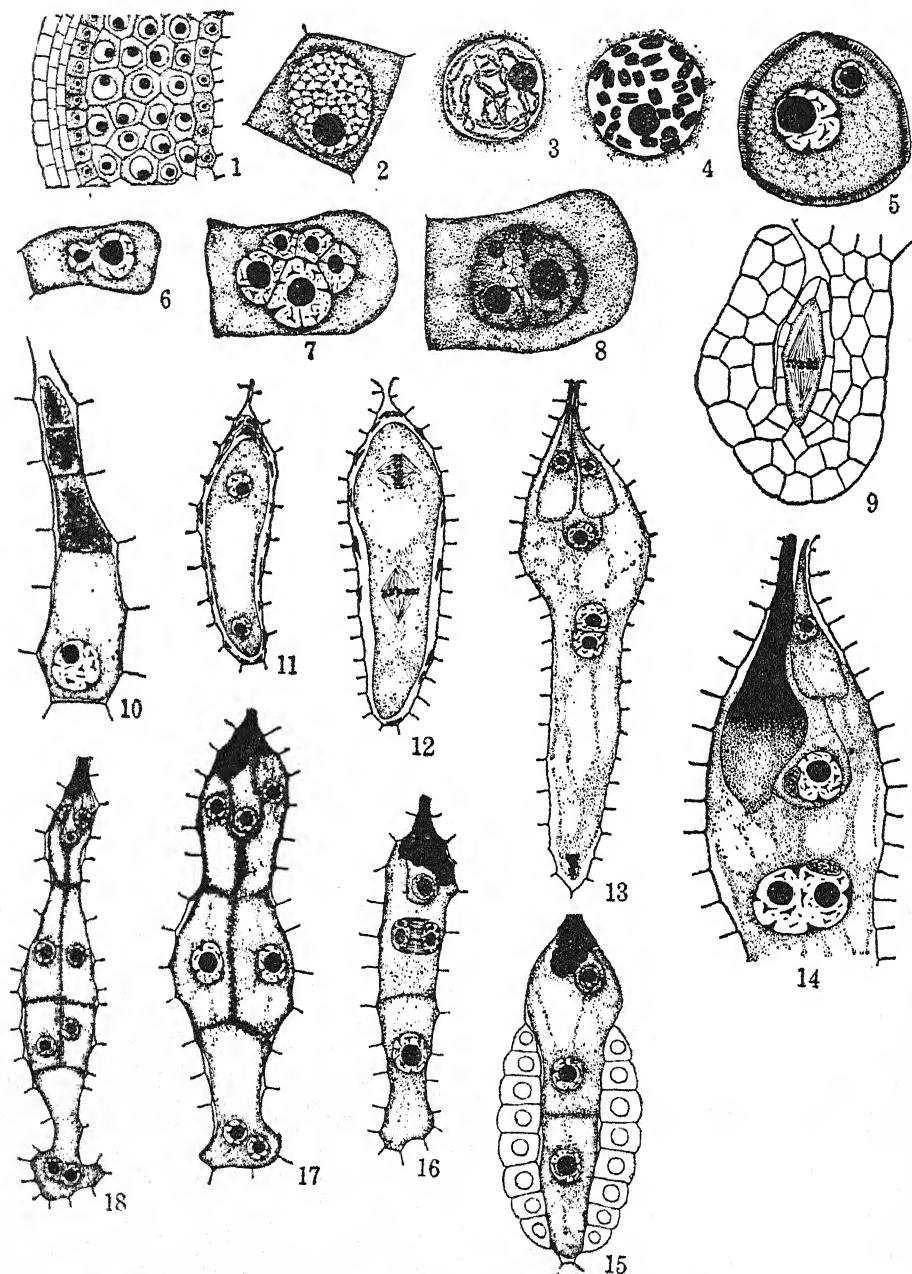
Figs. 1-29. *Didymocarpus tomentosa* Wt. Fig. 1. Portion of the anther showing the wall layers and the microspore mother cells (× 160). Fig. 2. Pollen mother cell with the nucleus in the resting stage (× 1095). Fig. 3. Pachytene stage in mother cell showing the paired chromosomes

innermost enlarges considerably and forms the tapetum. The middle layer is very much compressed by the enlargement of the fibrous and tapetal layers. The cells of the tapetum increase very much in size and their nuclei undergo divisions so that each cell becomes two- to five-nucleate. These nuclei, however, remain close together and later on, when the microspore mother cells are dividing, fuse to form one or two large deeply staining nuclear masses (Figs. 1, 6–8).

The usual meiotic changes have been observed in the microspore mother cells preceding their division (Figs. 2–4). Chromosome counts were made at diakinesis and 27 bivalents were observed (Fig. 4). The chromosome number for *Didymocarpus lavandulacea* is given as $X = 18$ and the basic number for the Cyrtandroideæ is held to be 9 (Sugiura, 1940). Thus the chromosome number obtained for *Didymocarpus tomentosa* is in agreement with the previous observations. As already recorded by Sugiura the chromosomes are small. The disposition of the two spindles in the second division may be either parallel or at right angles to each other. The microspores separate by the deepening of the peripheral furrows and are formed according to the simultaneous type.

The pollen grains are spherical and have three ridges. They are binucleate at the time of shedding as reported in *Gloxinia hybrida* (Elfving, 1879).

MEGASPOROGENESIS

The parietal placenta deeply protrudes into the single locule of the ovary and bears an indefinite number of anatropous ovules. The nucellar primordium arising on the placenta soon becomes invested by a single massive integument. The nucellus is much reduced and tapers towards the micropyle.

A single hypodermal cell at the summit of the nucellus forms the primary archesporium and can be recognised quite easily by its conspicuous size and deeply staining protoplasm. The primary archesporial cell directly functions as the megaspore mother cell which becomes much elongated. A linear tetrad of megaspores

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($\times$ 1460). Fig. 4. Diakinesis—27 bivalents are seen ($\times$ 1460). Fig. 5. Pollen grain at the time of shedding, showing the binucleate condition ($\times$ 1460). Fig. 6. A tapetal cell showing the fusion of two nuclei ($\times$ 1460). Fig. 7. A six-nucleate tapetal cell ($\times$ 1460). Fig. 8. A tapetal cell in the late stage showing the fused nuclei ($\times$ 1460). Fig. 9. First division in the megaspore mother cell. The reduced nucellus and the massive integument are also seen ($\times$ 525). Fig. 10. Linear tetrad showing the enlargement of the chalazal megaspore and the degeneration of the upper three ($\times$ 1095). Fig. 11. Two-nucleate embryo-sac, the nucellus showing degeneration ($\times$ 730). Fig. 12. The division stage in the formation of the four-nucleate embryo-sac ($\times$ 730). Fig. 13. Mature embryo-sac showing the egg-apparatus, the polar nuclei and the remains of the antipodals ($\times$ 730). Fig. 14. Portion of the embryo-sac at the time of fertilisation. The elongated synergid is seen protruding into the micropyle. The polar nuclei have just fused ($\times$730). Figs. 15, 16, 17 & 18. Stages in the formation of endosprm ($\times$ 730).

arises by the two divisions of the megaspore mother cell. The chalazal megaspore undergoes further development and gives rise to the embryo-sac while the upper three degenerate and are seen as dark masses (Fig. 10).

During the developmet of the embryo-sac the surrounding nucellar cells are seen in various stages of degeneration and finally disappear. The innermost layer of the integumet forms a conspicuous tapetum showing significant enlargement during the post-fertilisation stages.

EMBRYO-SAC

The development of the embryo-sac is normal. When ready for fertilisation, the embryo-sac is eight-nucleate. The micropylar end is dilated and lodges the egg apparatus. The integumentary tapetum does not surround this dilated portion, but terminates below it. The two synergids are conspicuous with their long tapering tips, which extend through the whole length of the micropyle (Figs. 13 and 14). The egg is of considerable size and is as usual vacuolate in its anterior end. The two polar nuclei lie in close proximity in the middle of the embryo-sac and fuse only at the time of fertilisation. The antipodals which are very small begin to degenerate very early and in many cases even their remains are not seen in the mature embryo-sac. The cells in the neighbourhood of the sac show degeneration owing to the activity of the latter.

In *Klugia zeylanica*, Schnarf (1921) records that the ends of the synergids lie immediately near the opening of the micropyle a condition also met with in *Didymocarpus*. In *Roettlera* sp. Glišic' (1934) mentions that in many cases the embryo-sac grows out of the micropyle with the 'synergids wandering into the bladder-like portion'. Oehlkers (1923) also observed a similar situation in *Monophyllæa Horsfieldii*. But in *Didymocarpus*, the embryo-sac does not show any tendency towards extra-micropylar development. In *Haberlea rhodopensis* Glišic' (1928) noted, in a few instances, the presence of two embryo-sacs in the same ovule. Such abnormalities have not been found in *Didymocarpus*.

FERTILISATION

One of the synergids is destroyed by the entry of the pollen tube. The other can be recognised for some time after fertilisation by the side of the pollen tube. The tip of the pollen tube is seen to be considerably dilated. The nucleus of the egg at the time of syngamy is seen to be in a resting condition. After the entry of the pollen tube into the embryo-sac, the two polar nuclei fuse prior to triple fusion (Fig. 14).

ENDOSPERM

The first division of the primary endosperm nucleus takes place in the middle of the embryo-sac and is followed by a transversely placed wall which separates the chalazal chamber from the

micropylar (Fig. 15).   The next division is longitudinal and occurs only in the micropylar chamber (Fig. 16).   The two micropylar cells thus formed undergo transverse divisions resulting in two tiers of two cells each (Fig. 17).   The terminal tier develops into the micropylar haustoria, while the inner gives rise to the endosperm by further divisions.   The margin of the endosperm in the later stages appears wavy in section due to the unequal enlargement of the tapetal cells (Fig. 29).   In the mature seed, we find the endosperm differentiated into three regions—the massive storage region composed of large cells filled with starch, and the smaller richly protoplasmic cells towards the ends forming the isthmus between the haustoria and the endosperm proper (Fig. 29).

HAUSTORIA

Only a single nuclear division takes place in the chalazal chamber.   The resulting binucleate haustorium is very aggressive and digests the tissue surrounding it until it reaches the epidermis of the integument.   The nuclei enlarge very much and become pear-shaped, as in *Roettlera* sp.   Further development of the haustorium is lateral giving it an anchor-like appearance in section (Fig. 22).   The remnants of absorbed cells can be seen about the haustorium.

The two cells cut off towards the micropyle remain uninucleate and develop into the micropylar haustoria.   These are also aggressive and eat their way into the surrounding tissue (Fig. 21).   At the region where they are connected with the endosperm, the cells of the integumentary tapetum are much enlarged, with their inner walls conspicuously thickened (Fig. 19).   A similar feature has been described by Glišic' (1934) in *Roettlera* sp.

In the later stages of their development the two micropylar haustoria are considerably elongated and extend through the whole length of the micropyle.   At times the terminal portions of these cells grow out of the micropyle and form a bulbous swelling into which the nucleus migrates (Figs. 19 and 20).   Thus, during later stages the haustoria are extra-micropylar.   As already mentioned, Schnarf and Glišic' have observed an extra-micropylar embryo-sac in *Klugia zeylanica* and *Roettlera* sp.   Although the embryo-sac in *Didymocarpus* does not show any extra-micropylar development, the haustorial cells develop beyond the micropyle.

The development of the endosperm in *Didymocarpus tomentosa* is seen to be of the ' *Brunella type* ' of Schnarf (1917).   A similar type of endosperm formation is reported in *Corytoloma cyclophyllum* (Laurent, 1917), *Haberlea rhodopensis* (Glišic', 1928) and *Roettlera* sp. (Glišic', 1934).   In *Klugia zeylanica*, Schnarf (1921) observed a different type of endosperm development.   In this case the second division taking place in the micropylar chamber is also transverse. A similar situation is also met with in *Ramondia Nathaliæ* and *R. Serbica* (Glišic', 1924).
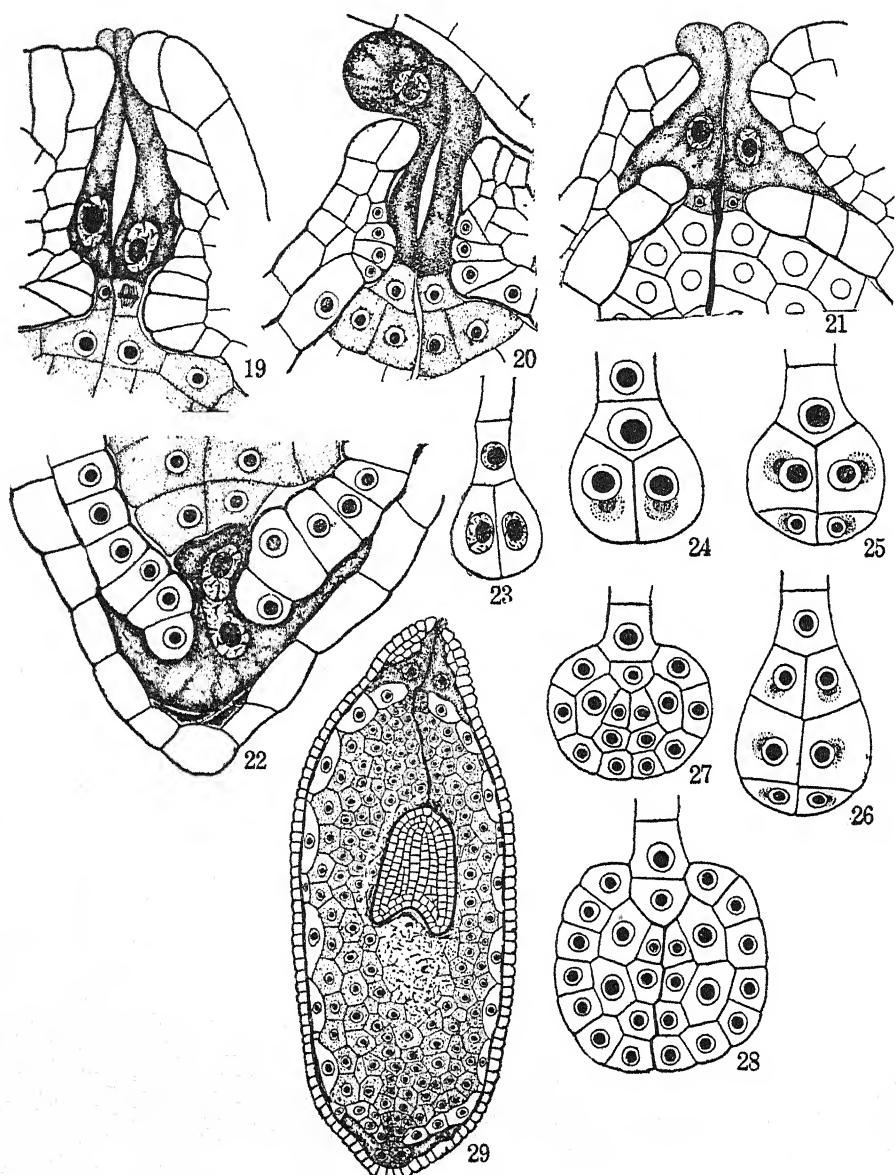
Figs. 19, 20 & 21. Stages in the enlargement of the micropylar haustoria showing their extramicropylar development. (Figs. 19 and 20. × 480 and Fig. 21 × 320.) Fig. 22. Chalazal haustorium showing the two hypertrophied nuclei (× 480). Figs. 23–28. Stages in the development of embryo (Figs. 23–26 × 730; Figs. 27 and 28, × 525). Fig. 29. Long section of a seed showing the enlarged tapetal cells and the embryo (× 130).

In the history of the chalazal haustorium, the two-celled stage often noticed in *Haberlea rhodopensis* (Glišic', 1928) is derived from a stage with a larger number of cells. The next step is the disappearance of the wall between the two cells, thus rendering the chalazal haustorium unicellular. but binucleate. This condition is seen in *Klugia Notoniana, Roettlera* sp., and normal cases of *Haberlea rhodopensis*. It is found that in *Didymocarpus* also the same condition prevails. The uninucleate condition of the chalazal haustorium is derived from the binucleate one, by the suppression of a nuclear division as in *Klugia zeylanica* and the two species of *Ramondia*.

While variations have been recorded in the nature of the chalazal haustorium, in all the plants of Gesneriaceæ studied so far, the micropylar haustoria are two in number and uninucleate. Their extra-micropylar development has been seen for the first time in *Didymocarpus tomentosa*.

EMBRYO

While the endosperm is being formed, the oospore elongates very much into a tubular structure. Further development begins only after the endosperm is considerably formed. The first division is transverse and separates the embryonal cell from the primary suspensor cell. By the divisions of the latter, a long suspensor of 8–10 cells is formed. The first and second divisions in the embryonal cell are longitudinal resulting in four cells (Figs. 23 and 24). The next division is transverse and of the eight cells at this stage the four cells towards the suspensor are larger than the distal ones (Fig. 25). The development of the embryo conforms to the *Capsella*-type (Figs. 26–28).

CONCLUSION

In evolution, the Gesneriaceæ are derived, in common with the parasitic Orobancheæ, form the Scrophulariaceæ. Both Gesneriaceæ and Orobancheæ possess a unilocular ovary with intruding parietal placenta bearing a large number of ovules. Both in the development of endosperm and haustoria, the two families show greater affinity to Scrophulariaceæ than to each other. The development of endosperm in members of Gesneriaceæ till now studied is of two types. The ' *Brunella*-type ' is seen in *Klugia Notoniana, Corytoloma, Roettlera* and *Haberlea*. On the other hand, the formation of endosperm in *Klugia zeylanica* and *Ramondia* belongs to *Paulownia*-type (Millsaps, 1936).

The extra-micropylar development of the haustoria seen in *Didymocarpus* is reported in several allied families. In *Orobanche Hederæ* (Glišic', 1929) the haustoria are to a certain extent extra-micropylar. This condition, however, is more pronounced in *Utricularia vulgaris* (Wylie and Yocom, 1923) and *U. cærulea* (Kausik, 1938). In the Scrophulariaceæ the development of the haustoria beyond the micropyle is noticed in several members like *Lathræa squamaria* (Glišic', 1932), *Vandellia* (Krishna Iyengar, 1940 *a*) and *Torenia* (Krishna Iyengar, 1941 *b*).

Summary

Meiosis in the microspore mother cells is normal. Chromosome counts at diakinesis reveal 27 bivalents. This agrees with the basic number 9 recorded for Cyrtandroideæ by Sugiura. The microspore formation is of the simultaneous type and the pollen grains are binucleate at the time of shedding.

The tapetal cells are found to be 2–6 nucleate, the nuclei fusing again during later stages.

The single hypodermal archesporial cell in the nucellus functions directly as the megaspore mother cell and gives rise to a linear tetrad of which the innermost megaspore develops into the embryo-sac.

The synergids show very long tips.

The first three divisions of the primary endosperm nucleus result in the organisation of the two micropylar haustorial cells, the endosperm proper in the middle, and the chalazal chamber which develops into an aggressive binucleate haustorium. The two uninucleate micropylar haustoria are also aggressive and grow beyond the micropyle.

The development of the embryo proceeds according to the *Capsella*-type.

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PUCCINIA PHYLLOCLADIAE COOKE, A NEW RECORD FOR INDIA

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(Communicated by H. Chaudhuri)

In April 1940 the writer collected at Lahore a rust on *Asparagus gracilis* Royle which seems to be a new record for India. The uredial stage was in full vigour and continued to be so in May and June ; the telial stage became manifest in July and continued until August. No other stage has been so far observed. The rust was later seen in the Jallo Forests near Lahore.

The urediosori are chiefly on the stem and the branches, a few having been formed on the phylloclades also. The sori are broad at the centre and taper towards the ends and are principally aggregated into large brown patches near the base of the plant. The urediospores are pale brown, elongated or ellipsoid, $18-40 \times 14-20 \mu$, pedicillate, the stalk being deciduous and $26 \times 4 \mu$ in length ; the epispore is minutely echinulate with 2–5 germ pores which are hyaline and situated along the equatorial line.

The telia are present all over the plant but chiefly on the stem ; they are often aggregated to form black patches running along the entire stem. The teliospores are deep brown, subglobose to oblong, with rounded apex and base. They measure $33-48 \times 22-30 \mu$, pedicillate, stalk being persistent, pale brown and $11-137 \times 3-10 \mu$; epispore is smooth, dark brown, thickened all round, $4-6 \mu$ thick ; the septum between the two cells is upto 7μ thick and much darker in colour.

Unicellular, dark brown, pedicillate, obtuse, ovate to obovate mesospores were observed within the telial sori. They measure $26-44 \times 22-26 \mu$ and their apex is upto 13μ thick. The stalk is persistent and brownish. There is no germ pore in the mesospores.

The rust belongs to the genus *Puccinia* of which three species have so far been described on *Asparagus* : *Puccinia Asparagi* De., *Puccinia Asparagi-lucidi* Diet., and *Puccinia phyllocladiæ* Cooke. The Lahore specimen agrees most with the last named species which has not so far been reported to occur in India.

Puccinia phyllocladiæ was described by Cooke (1882) on *Asparagus falcatus* L. from Natal and a second collection was made by Thwaites in Ceylon, according to Sydow (1904). Petch (1912), however, was not able to find any Ceylon specimens collected by Thwaites at Kew or at Peradineya. He himself collected it at Hakgala, Ceylon, in 1910. Cooke (1882) and Sydow (1904) have

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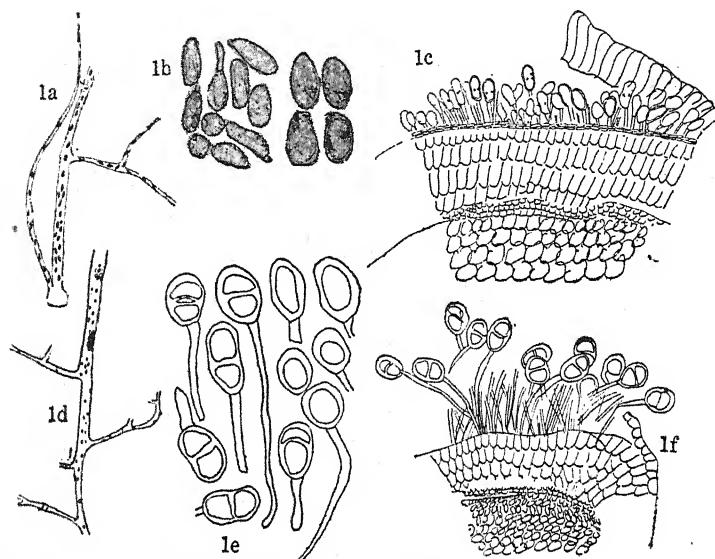
Text-fig.—1. *Puccinia phyllocladiæ* on *Asparagus gracilis.* (*a*) Infected branch with urediosori (× ½). (*b*) Urediospores (× 285 ; 350). (*c*) T.S. of stem passing through a urediosorus (× 170). (*d*) Infected branch with teliosori (× ½). (*e*) Teliospores (× 285). (*f*) T.S. of stem passing through a teliosorus (× 170).

described only the telial stage but the æcial and the uredial stages were for the first time described by Petch (1912). The writer was not able to find the æcial stage but he has found not only the uredial and the telial stages but the mesospores as well.

The Lahore specimen has slightly smaller urediospores than those reported by Petch (*loc. cit.*) but the teliospore measurements more or less agree. The pedicel of the teliospores of the writer's specimen is very long and agrees in this respect with the Natal specimen. In spite of these few differences in the measurements there is little doubt that the fungus on *Asparagus gracilis* Royle is *Puccinia phyllocladiæ* Cooke. Specimens have been deposited in the Herb. Crypt. Ind. Orient. of the Imperial Agricultural Research Institute, New Delhi, and in the Herbarium of the Botanical Department of the Panjab University, Lahore.

The writer expresses his thanks to Dr. H. Chaudhuri under whose direction the work was done and to Dr. B. B. Mundkur of the Imperial Agricultural Research Institute, New Delhi, for help given in reading the manuscript of the paper.

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KARTAR SINGH THIND—

THE GENUS PERONOSPORA IN THE PUNJAB

EXPLANATION OF COLOURED FIGURES

All drawn to half natural size.

All drawn to half natural size.

THE GENUS *PERONOSPORA* IN THE PUNJAB

By Kartar Singh Thind, M.Sc.

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(Communicated by H. Chaudhuri)

COLLECTIONS of plants attacked by the downy mildews were made by the writer in the Punjab, chiefly in the districts of Lahore, Amritsar, Lyallpur and Jullundur in the months of January to April 1940. Twenty hosts in these collections were attacked by species of the genus *Peronospora* and this paper deals with their taxonomy.

These forms on the 20 hosts comprise 16 species of which 6 are new records for India ; 7 of the hosts are also new records. A species of *Peronospora* on *Malcolmia africana* is being reported, as far as the writer has been able to find out, for the first time. It has been tentatively placed in *Peronospora Brassicæ* Gäumann.

The genus *Peronospora* Corda has been very extensively studied by Gäumann (1923) and his monograph will continue to be of great help to all investigations of this genus for a long time to come. It brings together in a single comprehensive publication much of the published literature on the genus and a good deal of information on the Exsiccatæ issued by the mycologists in the past. There is considerable controversy, however, regarding Gäumann's attempts to segregate into new species some of the composite species of the older authors. These new species have many times been established merely on the basis of their host specificity, there being hardly any morphological differences to distinguish them from one another. Field observations confirm, however, the high degree of parasitic specialization which the forms possess. Whether these forms deserved specific rank on that account or one should have been satisfied with new varieties is a point which further research on the genus alone can decide. For the present, the writer has accepted Gäumann's conclusions following the lead given by Savulescu *et al.*, Sydow (1923), Bisby (1938), Mundkur (1938) and others.

The specimens which formed the basis of this study have been deposited in the Herbarium of the Botanical Laboratory, Panjab University, Lahore, and Herbarium Cryptogamæ Indiæ Orientalis of the Imperial Agricultural Research Institute at New Delhi.

Peronospora SPECIES ON THE Leguminosæ

1. *Peronospora Viciæ-sativæ* Gäumann, *Beit. Kryptogam. Schweiz.*, V., p. 219, 1923.

Host.—*Vicia-sativa* L.

Loc.—Very Common ; abundant in March–April.

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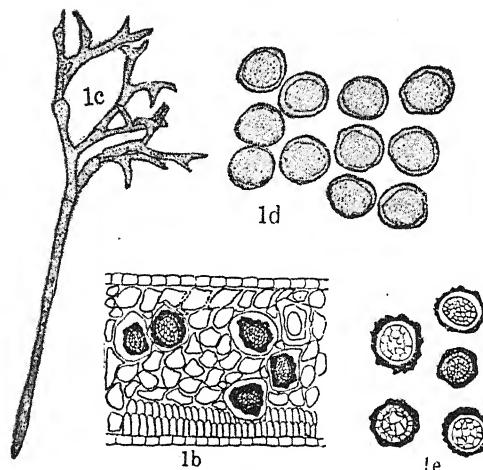
Fig. 1. *Peronospora Vicia-sativæ* on *Vicia sativa*. Fig. 1 b. T.S. of the infected leaf with oospores (× 175). Fig. 1 c. Conidiophore (× 420). Fig. 1 d. Conidia (× 280). Fig. 1 e. Oospores (× 285).

Symp.—Characterised by the formation of deep reddish pink spots or patches on both sides of the leaf and also on branches; growth appeared cottony and grey violet.

Conidiophore.—Emerging in small groups through stomata on the undersurface, 5–7 times dichotomously branched 163–596 μ long; trunk long, erect, 26–448 × 11 μ; crown of branches at the top covering one-third of the total length of the trunk; primary branches straight or slightly curved, upper ones curved and spreading; ultimate branchlets situated at obtuse angle (or right angle), pointed, unequal, short, upto 18 × 2–3 μ.

Conidia.—Borne singly at the tips of ultimate branchlets, similar at both ends (rarely narrowed towards one end) obtuse or subobtuse but chiefly oval, pale greyish brown, 15–30 × 15–20 μ.

Oospores.—30–37 μ in diameter, deep pink or yellow; oogonial wall persisting and wrinkled; epispore deep yellow or deep pink, rough, irregular, spongy, with projections; exine smooth, completely circular, pale yellow to pinkish, imparting pinkish colour to the infected parts.

2. *Peronospora Lathyri-palustris* Gäumann, *Beitr. Kryptogamenfl. Schweiz.*, V, p. 192, 1923.

Host.—*Lathyrus sativus* L.

Loc.—Lahore (near Shahdra); Common in April.

Symp.—Grey violet patches all over the plant.

Conidiophore.—Emerging through stomata or otherwise in small groups, 204–603 μ long, 5–6 times dichotomously branched; trunk stout, upto 418 × 4–11 μ; branches cover only three-fourths of the
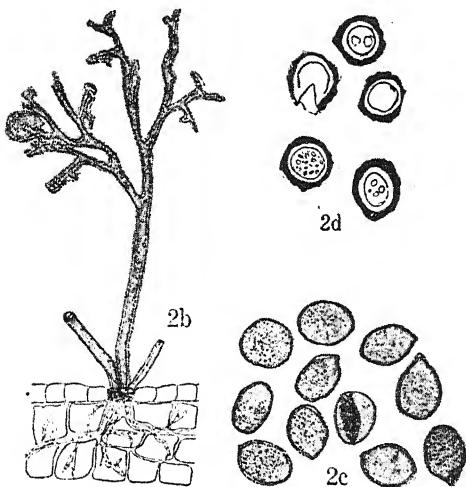
Fig. 2.   *Peronospora Lathyri-palustris* on *Lathyrus sativus*.   Fig. 2 *b*.
A part of T.S. of the infected leaf showing haustoria and a fascicle of 3
conidiophores coming out through a stoma (× 140).   Fig. 2 *c*.   Conidia
(× 280).   Fig. 2 *d*.   Oospores (× 285).

trunk ; primary branches straight going upwards and outwards ;
secondary branches more spreading ; ultimate branchlets diverging
at obtuse angles, unequal, tapering and sharp-pointed, cruved, upto
22 μ long.

Conidia.—Sub-globose to broadly elliptic with no difference
between base and apex, rarely broader at one end and narrow at the
other, wall black, contents blackish brown, 15–29 × 15–21 μ.

Oospores.—26–45 μ in diameter ; oogonial wall persistent ; epi-
spore spongy, rough, irregular, deep pinkish brown ; exine smooth,
spherical and light pink.

  3.   *Peronospora Trigonella* Gäumann, *Beitr. Kryptogamenfl.
        Schweiz.*, V, p. 216, 1923.

Host.—*Trigonella fœnum-grœcum* L.

Loc.—Very common in the Punjab from February to March.

Symp.—Infected leaves readily recognised by the presence of
yellow patches on their upper surface ; at the corresponding area on
the under-surface, cottony, grey-violet, small patches were formed ;
patches scattered all over the leaf surface.

Conidiophores.—Robust, emerging through stomata in small
fascicles, 5–8 times dichotomously branched, 389–551 μ long ; trunk
well represented, stout, erect or rarely oblique, 207–365 × 8–14 μ ;
branches covering three-fourths the length of the trunk ; primary
branches straight, intermediate ones bent but not spreading ; ulti-
mate branchlets in pairs, small, sharp-pointed, usually equal, at right
angles or obtuse angle, upto 37 μ.
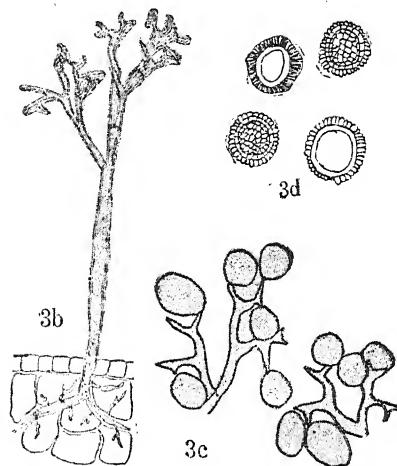
Fig. 3. *Peronospora Trigonella* on *Trigonella fœnun-græcum*. Fig. 3 *b*. A portion of T.S. of infected leaf showing haustoria and a conidiophore coming out (× 140). Fig. 3 *c*. Conidia (× 280). Fig. 3 *d*. Oospores (× 285).

Conidia.—Broadly ellipsoid, narrow towards one end and broad at the other, wall distinctly black, contents slightly greyish brown, 19–34 × 15–22 μ.

Oospores.—30–45 μ in diameter; oogonial wall persistent and wrinkled; epispore deep brown, irregular, with projections, thick and typically spongy; exine completely circular, pale whitish grey, smooth.

4. *Peronospora æstivalis* Sydow, in Gäumann, *Beitr. Krypto-gamenfl. Schweiz*, V, p. 200, 1923.

Host.—*Medicago sativa* L. and *M. denticulata* Wiled.

Loc.—Lahore, Amritsar and Jullundur; January to March.

Symp.—Entire lucrine crop pale and meagre in appearance; downy patches of blackish grey colour forming thick layers on entire under-surface of the leaves, petioles and stems; plants with pale and yellow appearance. Infection on *M. denticulata* very slight and characterised by reddish streaks on the upper-surface and grey downy patches on the under-surface.

Conidiophores.—Emerging through stomata in groups of 1 to 5 or more, 5–8 times dichotomously branched, 174–462 μ long; trunk straight, stout, bulbous, 81–278 × 8–11 μ; branched portion 78–189 μ long or three-fourths of the entire trunk; primary branches straight or oblique, intermediate ones curved in centre and situated at an acute angle; ultimate branchlets unequal, each pair at an angle from acute to straight, 4–26 × 2–3 μ.
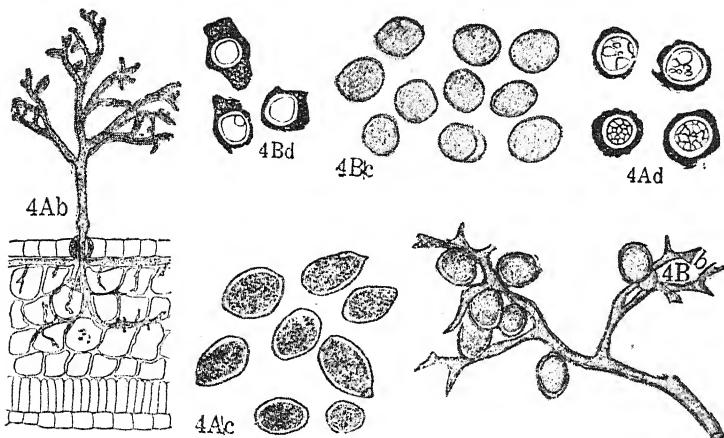
Fig. 4. *Peronospora æstivalis* on :—A. *Medicago sativa* : Fig. 4 *b*.
T.S. of the infected leaflet showing haustoria and coming out of a
conidiophore through a stoma (×140). Fig. 4 *c*. Conidia (×280). Fig. 4 *d*.
Oospores (× 285). B. *Medicago denticulata* : Fig. 5 *b*. A part of coni-
diophore (× 420). Fig. 5 *c*. Conidia (× 280). Fig. 5 *d*. Oospores
(× 285).

Conidia.—Sub-globose to broadly ellipsoid, narrowed towards
one end and broader at the other, contents bright or dirty brown,
wall blackish 22–37 × 15–26 μ.

Oospores.—Late forming, buried inside the tissue ; oogonial
wall persistent and wrinkled ; epispore thick, rough, irregularly
rounded, deep brown ; exine circular, smooth, pale or pale yellowish
grey to bright, contents granular or greyish, 22–45 μ in diameter.

Aggressively parasitic on the host *M. sativa* only.

5. *Peronospora Trifolii-repentis* Sydow in Gäumann, *Beitr.*
Kryptogamenfl. Schweiz., V, p. 215, 1923.

Host.—*Trifolium resupinatum* L.

Loc.—Common everywhere in February and March.

Symp.—Downy patches scanty, principally on the under-
surface of the leaflets, in the form of long tufts parallel to the vein-
lets which act as boundaries, brown streaks at corresponding places
on the upper-surface towards the apex or along the margin.

Conidiophores.—Robust, emerging through stomata, singly or
at the most in twos, 5–8 times dichotomously branched, 258–681 μ
long ; trunk 81–432 × 8–11 μ, stout, prominent ; branches two-thirds
the length of the trunk ; primary branches straight, spreading, inter-
mediate ones straight, rarely curved inwards, interlocking ; ultimate
branchlets unequal, situated at right angles, upto 22 μ long, sharply
pointed, curved both inwards and outwards, smaller one in each pair
often like a mere projection.
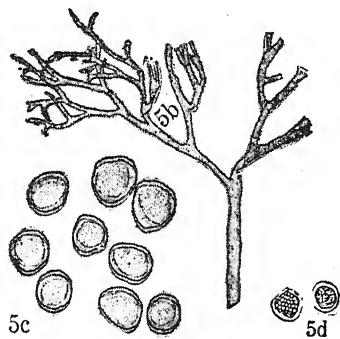
Fig. 5. *Peronospora Trifolii-repentis* on *Trifolium resupinatum.*
Fig. 5 *b.* Conidiophore (× 140). Fig. 5 *c.* Conidia (× 280). Fig. 5 *d.*
Oospores (× 285).

Conidia.—Obtuse or subobtuse, no difference between base and apex; wall darkish, contents light brown, 19–30 × 17–22 μ.

Oospores.—Very scanty; oogonial wall present; epispore dirty brown, irregular; exine of usual type; 22–41 μ in diameter.

6. *Peronospora Meliloti* Sydow in Gäumann, *Beitr. Krypto-gamenfl. Schweiz.*, V, p. 203, 1923.

Hosts.—*Melilotus parviflora* Desf. and *M. alba* Desr.

Loc.—Common, February and March.

Symp.—Downy growth on under-surface nearer the apex of the leaflets, patches single or crowded, never covering the entire surface, corresponding areas on upper-surface pale on account of loss of chlorophyll.

Conidiophores.—Robust, emerging from stomata in groups of 2–5 or more, 5–7 times dichotomously branched, 237–502 μ long;
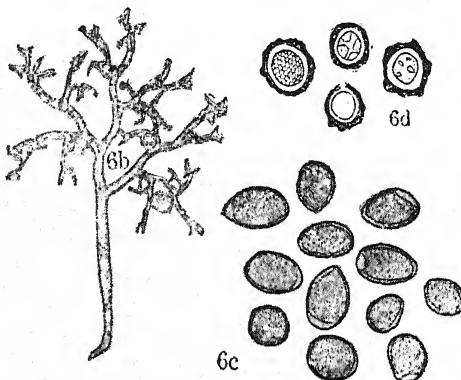
Fig. 6. *Peronospora Meliloti* on *Melilotus parviflora*. Fig. 6 *b.* Coni-diophore (× 140). Fig. 6 *c.* Conidia (× 280). Fig. 6 *d.* Oospores (× 285).

trunk 104–318 × 7–15 μ, bulbous at base ; branches covering two-thirds the length of the trunk ; primary branches straight, secondary spreading and recurved ; ultimate branchlets situated at obtuse angles, reflexed, unequal, tapering, blunt, sometimes parallel and diverging, 4–19 × 1–2 μ.

Conidia.—Given off singly, sub-globose to broadly elliptical, broader at one end and narrowed at the other, grey brown, 15–32 × 15–26 μ, one or more hyaline strips on the wall of conidia in a few cases.

Oospores.—19–35 μ in diameter ; oogonial wall persistent and wrinkled ; epispore thick, deep yellow, spongy, irregular, more or less circular ; exine completely circular, smooth, bright whitish grey.

7.   *Peronospora astragalina* Sydow in Gäumann, *Beitr. Krypto-gamenfl. Schweiz.,* V, p. 188, 1923.

Host.—*Astragalus tribuloides* Delile.

Loc.—Lahore, not common, March–April.

Symp.—Plants being hairy, small inconspicuous downy patches are made out only with difficulty.

Conidiophores.—Very little material was available. Conidio-phores robust, emerging through stomata in groups, 5–8 times dicho-tomously branched, 163–407 μ long ; trunk thick, erect upto 241 × 4–13 μ ; branches covering three-fourths the length of the trunk ;
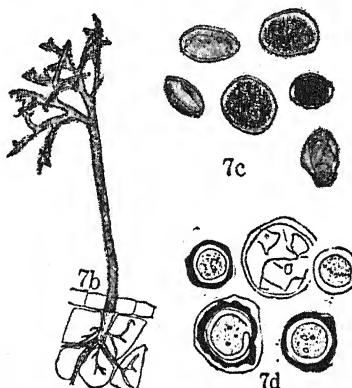
Fig. 7.   *Peronospora astragalina* on *Astragalus tribuloides.*   Fig. 7 *b.* T.S. of the leaf showing haustoria and coming out of conidiophore through a stoma (× 140).   Fig. 7 *c.* Conidia (× 280).   Fig. 7 *d.* Oospores (× 285).

primary branches straight, slightly extending outwards ; secondary ones spreading and curved ; ultimate branchlets curved in or out-wards at obtuse or right angle, equal, rarely unequal, tapering, sharp-pointed, thin, upto 20 μ long.

Conidia.—Ellipsoid, 19–26 × 15–19 μ, wall black and contents greyish brown, drab brown when young.

Oospores.—30–48 μ in diameter; oogonial wall persistent; epispore rough, irregularly rounded, deep brown; exine smooth, spherical, pale greyish brown, bright.

[*Note.*—There are two species of *Peronospora* recorded on *Astragalus* spp., viz., *P. astragalina* Sydow and *P. Astragali* Sydow. Latter has smaller conidia but no oospores. Conidial measurements obtained by the writer are slightly different from those given for the two species but on the whole the Punjab species agrees with the former and is characterised by abundant formation of oospores. Gäumann (*loc. cit.*) himself is not satisfied that the biological identity of the two species has been definitely proved.]

Peronospora Species on Chenopodiaceæ

8. *Peronospora effusa* (Grev.), Rabenhorst in *Herb. Myc.*, Ed. I, No. 1880 ; *Saccardo, Syll.* VII, p. 256, 1888.

Host.—*Chenopodium album* L.

Loc.—Common, February to March.

Symp.—Infected leaves with yellow coloured patches on upper-surface and downy grey-violet masses on lower surface; infection not wide spread and only a few leaves in a plant attacked.

Conidiophores.—Drab, emerging through stomata in groups of 2–8, 5–7 times dichotomously branched, 440–548 μ long; trunk stout,
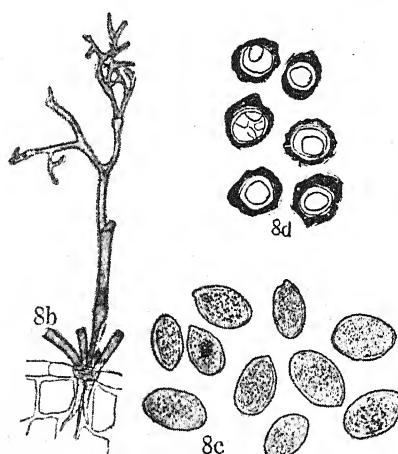
Fig. 8. *Peronospora effusa* on *Chenopodium album.* Fig. 8 *b.* A part of the T.S. of leaf showing a fascicle of conidiophores coming out through a stoma (× 140). Fig. 8 *c.* Conidia (× 280). Fig. 8 *d.* Oospores (× 285).

erect, 355×7–12 μ ; crown of branches covering three-fourths of the length of the trunk ; primary branches straight, directed outwards and then inwards ; secondary curved outwards and then inwards. sometimes all branches interlocked ; ultimate branchlets at an angle

from acute to right, running almost parallel, usually unequal, smaller ones generally in the form of hooks, tapering, blunted, upto $26 \times 4 \mu$.

Conidia.—Ovate-oblong or ellipsoid, broader at one end and narrower at the other, provided with prominent nipple towards the narrow end, brownish, 22–37×19–23μ.

Oospores.—Deep brown, darkish, 22–45μ in diameter ; oogonial wall present or not, brownish ; epispore thick, deep reddish brown or dark brown, more or less circular ; exine fairly greyish white, smooth, spherical.

(*Note.*—See remarks under *P. variabilis* Gäumann.)

9. *Peronospora variabilis* Gäumann. *Beitr. Kryptogamenfl. Schweiz.*, V, p. 226, 1923.

Host.—*Chenopodium album* L. ·

Loc.—Common, July to September.

Symp.—Downy mass thin as against a thick layer in the last case. Usually appears late. Brown spots on leaves.

Conidiophores.—Robust, emerging through stomata in groups of 1–6, 3–5 times dichotomously branched, upto 437μ long; trunk 311×8–11μ, erect or slightly oblique; branches generally spreading (rarely interlocking) as long as 126μ, *i.e.*, only 2/5 of the length
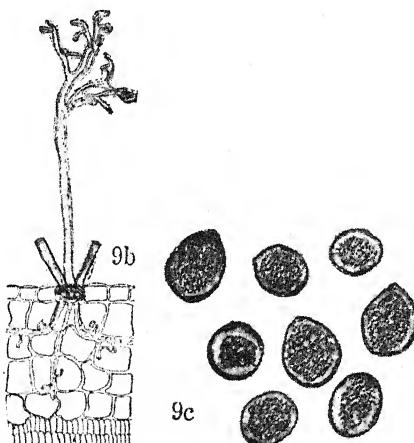
Fig. 9.   *Peronospora variabilis* on *Chenopodium album*.   Fig. 9 *b*.   T.S. of infected leaf showing haustoria and coming out of conidiophores through a stoma ($\times$ 140). Fig. 9 *c*. Conidia ($\times$ 280).

of the trunk; primary and secondary branches curved, usually spreading; ultimate branchlets unequal, pointed at acute to right angles, appearing like the open pairs of a forceps, upto 30μ long.

Conidia.—Broad, rarely pointed, usually globose to sub-globose, 24–33 × 19–30 μ and without a nipple.

Oospores not seen.

Remarks.—There is some confusion regarding the nomenclature of this fungus. Butler and Bisby (1931) state that the *Peronospora* on *C. album* is *P. effusa* and Mundkur (1938) places it in *P. variabilis.* Evidently both the mildews occur on this host. They differ in the following respects :—

P. effusa	P. variabilis
1. Appears early in February.	1. Appears late in July.
2. No. of conidiophores coming out through each stomata 2–8.	2. No. of conidiophores coming out through each stomata 1–6.
3. Conidiophores dividing 5–8 times.	3. Conidiophores dividing 3–5 times.
4. Conidiophores much longer up to 550 μ.	4. Conidiophores upto 457 μ long.
5. Conidia elliptical, elongated and pointed, provided with a distinct nipple, 22–33 × 19–23 μ.	5. Conidia broader and rarely pointed, usually globose or sub-globose, 24–33 × 19–30 μ, no nipple seen.
6. Oospore stage well developed.	6. Oospore stage not observed.

10. *Peronospora Kochiœ* Gäumann, *Mitt. Naturf. Ges. Bern.,* 1918 (1919), p. 64.

Host.—*Kochia indica* Wight.

Loc.—Lahore, March and April.

Symp.—Tips of infected leaves bleached ; small cottony grey-violet patches beneath the bleached areas ; disease not very common and caused little harm to attacked plants.

Conidiophores.—Robust, emerging in small numbers, 5–7 times dichotomously branched, 130–551 μ long ; trunk erect, 285 × 7–11 μ ; branches covering a length equal to that of the trunk ; primary branches straight and parallel, intermediate ones curved ; ultimate branchlets curved, pointed unequal, small, upto 20 μ long, diverting at an obtuse angle.

Conidia.—Obtuse to ellipsoid, tapering towards one side but not pointed ; drab brown when young, greyish brown when old, wall black, 22–37 × 15–19 μ, without a nipple.

Oospores.—30–37 μ in diameter ; oogonial wall brownish ; epispore thick, rough, wrinkled, deep brown ; exine smooth, spherical, drab white.
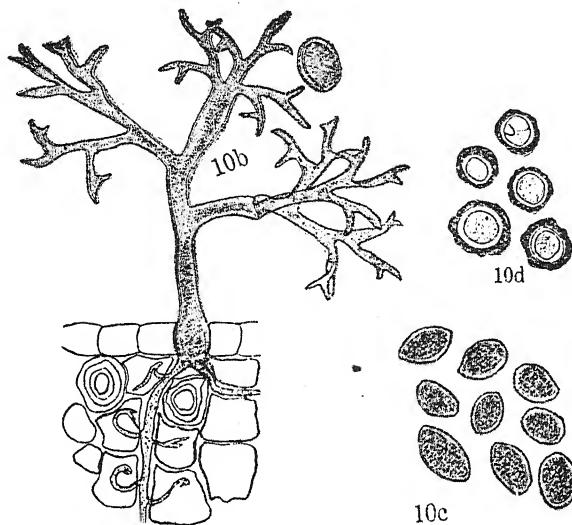
Fig. 10. *Peronospora Kochiæ* on *Kochia indica*. Fig. 10 *b*. A part of the T.S. of the infected leaf showing haustoria, oospores and coming out of a conidiophore (× 280).   Fig. 10 *c*.   Conidia (× 280).   Fig. 10 *d*. Oospores (× 285).

[*Note*.—Gäumann (1918) described *P. Kochiæ* on *Kochia sedoides* Scwad. and the species has so far been reported only from Russia.   He did not find oospores which have been for the first time recorded and described here.]

Peronospora SPECIES ON Papaveraceæ

11.   *Peronospora arborescens* (Berk.) de Bary, *Ann. Sci. Nat.*, Ser. 4, XX, p. 119, 1863.

Host.—*Papaver Rhoeas* L.

Loc.—Lahore, March to April.

Symp.—Infected areas withered and whole leaves covered with pale brown spots which are abundant and aggregated towards the apex and margins of leaves ; grey-violet downy growth scattered over whole of the under-surface of the leaves.

Conidiophores.—Emerging through stomata in small groups, 4–7 times dichotomously branched, robust, sub-erect, 204–389 μ long ; trunk cylindrical, elongated, 93–270 × 4–11 μ ; crown of branches covering half the length of the trunk ; primary branches straight, secondary and the upper ones flexuous and rarely spreading ; ultimate branchlets curved small, situated at acute or right angle, rarely obtuse, recurved, sharp-pointed upto 41 μ long.
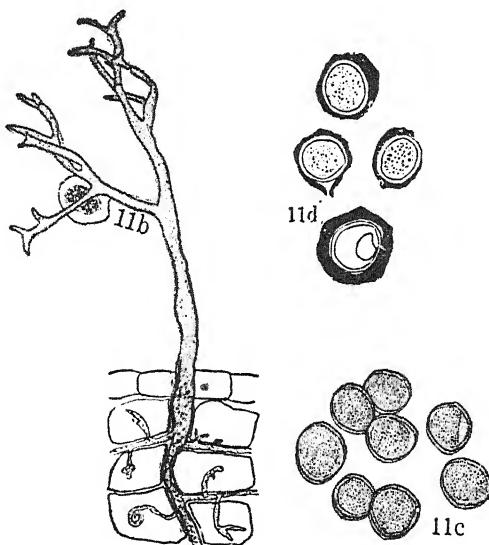
Fig. 11. *Peronospora arborescens* on *Papaver Rhoeas*. Fig. 11 *b*. Part of the T.S. of the infected leaf showing haustoria and coming out of a conidiophore (× 280). Fig. 11 *c*. Conidia (× 280). Fig. 11 *d*. Oospores (× 285).

Conidia.—Greyish brown, globose to sub-globose or ellipsoid, never pointed, 19–33 × 19–22 μ.

Oospores.—26–52 μ in diameter, spherical ; oogonial wall persistent, drab brown ; epispore deeply drab brown, irregular ; exine pale, faintly greyish brown, bright, smooth, completely spherical.

Peronospora SPECIES ON Rubiaceæ

12. *Peronospora Aparines* (de Bary). Gäumann, *Svensk. Bot. Tidskr.*, XII, p. 444, 1919.

Host.—*Galium Aparine* L.

Loc.—Fatehgarh, Mian di Khui (Lahore Dist.), Attari (Amritsar Dt.), March to April.

Symp.—Infected leaves turn pale, grey-violet downy growth on the under-surface of the apical part of the leaves. Leaves being hairy, the minute downy patches can be made out only by careful observation.

Conidiophores.—4–7 times dichotomously branched, robust, 93–481 μ long ; trunk thick, erect, or oblique, 37–293 × 6–15 μ ; primary branches given off at acute angle ; intermediate ones slightly bent or spreading ; ultimate branchlets small, pointed, curved, thin, unequal, 5 μ long.
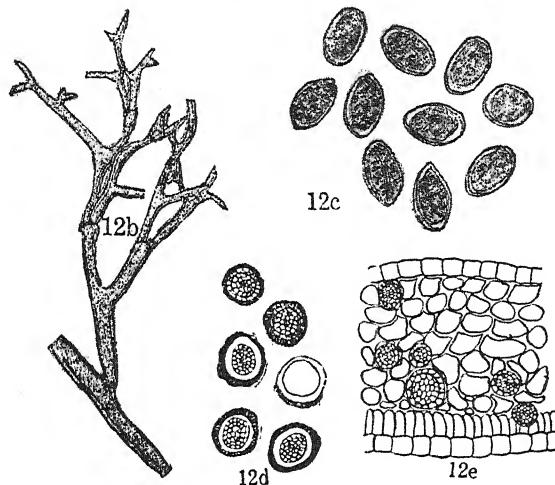
Fig. 12.   *Peronospora Aparines* on *Galium Aparine*.   Fig. 12 *b*.   A part of conidiophore (× 420).   Fig. 12 *c*.   Conidia (× 280).   Fig. 12 *d*.   Oospores (× 285).   Fig. 12 *e*.   T.S. of infected leaf with oospores (× 175).

Conidia.—Elliptical or globose, similar on both sides, sometimes one end broader, pale brown or drab brown when young, 19–37 × 15–20 μ.

Oospores.—Well developed, 22–40 μ in diameter ; oogonial wall persistent, irregular, wrinkled, epispore deep brown, characteristically spongy ; exine smooth, pale brown, bright, completely spherical.

Peronospora SPECIES ON Cruciferæ

13.   *Peronospora Brassicæ* Gäumann, *Beitr. Bot. Centr.*, XXXV, Abt. 1, p. 131, 1918 and *Beitr. Krypt. Sch.*, V, p. 260, 1923.

Hosts.—*Brassica campestris* L. ; *Brassica napus* L. ; *Raphanus sativus* L. ; *Malcolmia africana* Br.

Loc.—Common all over.   January to April.

Symp.—Infected areas bleached and raised on upper-surface in a few, corresponding areas on the under-surface possess white cottony patches scattered all over.   Infection on *Malcolmia africana* was more pronounced.

Conidiophores.—On *B. campestris* : Robust, emerging through stomata in small groups, 5–7 times dichotomously branched, 240–570 μ long ; trunk stout, erect or oblique, bulbous at the base, 141–422 × 15–19 μ ; branches forming a triangular compact mass covering one half of the trunk ; primary branches straight or spreading, nearly as thick as the trunk ; secondary or upper branches curved and often
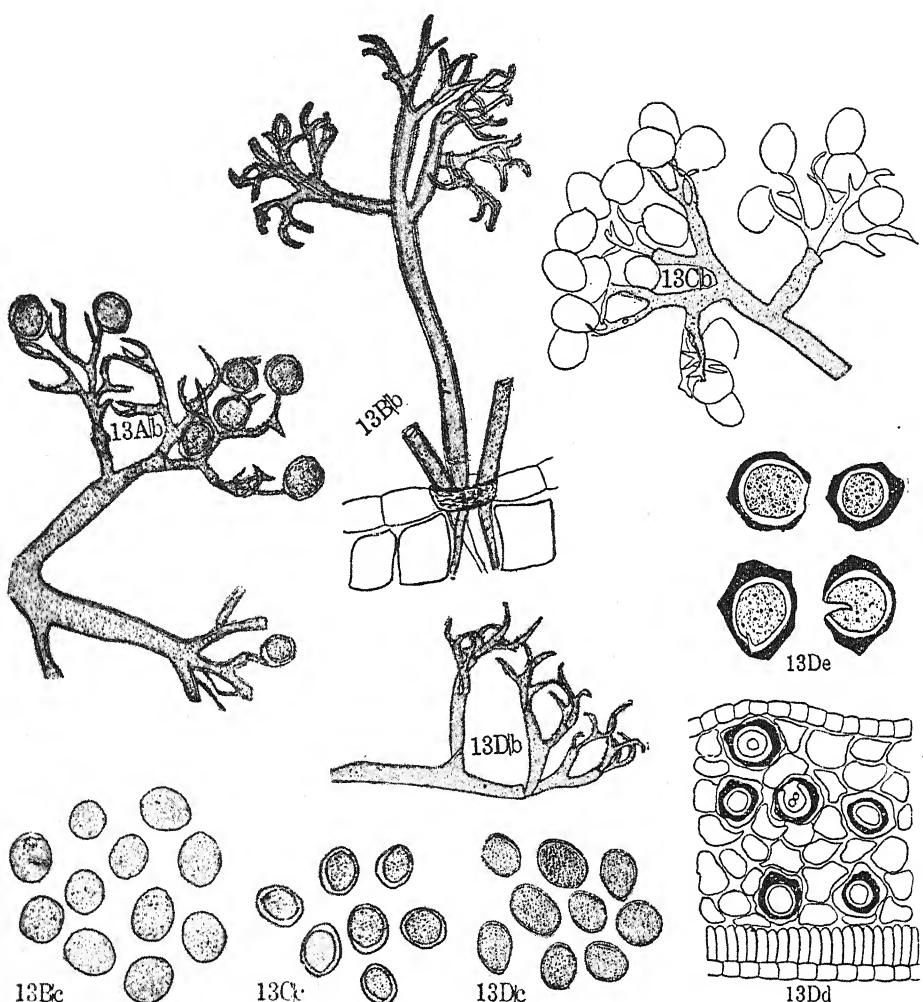
Fig. 13. *Peronospora Brassicæ* on :—A. *Brassica campestris*: Fig. 13 *b*. Conidiophore with conidia (× 420). B. *Brassica napus*: Fig. 13 *c*. A fascicle of conidiophores coming out of a stoma (× 280). Fig. *c*. Conidia (× 280). *C. Raphanus sativus*: Fig. 1 *b*. A part of conidiophore (× 420). Fig. *c*. Conidia (× 280). D. *Malcolmia africana*: Fig. *b*. A part of conidiophore (× 420). Fig. *c*. Conidia (× 280). Fig. *d*. T.S. of the infected leaf with oospores (× 175). Fig. *e*. Oospores (× 285).

fastly interlocked ; ultimate branchlets thin, pointed sharply, equal, hooked at the tip, upto 30 μ long.

On *B. napus* : Robust, coming out through stomata in small groups, 5–8 times dichotomously branched, 74–625 μ long ; trunk straight or slightly oblique, 26–440 × 8–20 μ ; branches forming more or less a triangular mass and covering one third of the length

of the trunk ; primary branches and utlimate branchlets similar as in the last.

On *Raphanus sativus* : Drab brown, coming out through stomata in groups 5–7 times dichotomously branched, 148–537 μ long ; trunk erect or oblique, 43–370 × 11–19 μ ; branches forming more or less a compact triangular mass ; covering about one half of the length of the trunk ; lower as well as upper branches similar as in the last.

On *Malcolmia africana* : Robust, coming out through stomata in large groups, upto 7 times dichotomously branched, 222–489 μ long ; trunk thick ; erect or slightly oblique, 130–378 × 14–19 μ ; branches covering about one third of the length of the trunk ; primary branches straight and going outwards, upper branches more curved and fastly interlocked ; ultimate branchlets thin, sharp-pointed, equal, running parallel like the pairs of a forceps upto 30 μ long.

Conidia.—In form, shape and colour, they are similar in all cases, differing only slightly in dimensions. Obtuse to sub-obtuse, both ends similar, robust or drab brown ; 15–20 × 15–19 μ in *B. campestris* ; 22–28 × 22–24 μ in *B. napus* ; 11–26 × 11–19 μ in *Raphanus sativus* ; 19–26 × 15–19 μ in *Malcolmia africana*.

Oospores.—Observed only in *Malcolmia africana* in March. 37–56 μ in diameter ; oogonial wall persistent, drab brown ; epispore deep drab brown, thick, irregular ; exine smooth, pale greyish, completely spherical.

14. *Peronospora parasitica* (Pers.) de Bary, *Rech. sur. Ann. Sci. Nat.*, 1863, Ser. 4, t. XX, p. 110.

Host.—Eruca sativa Mill.

Loc.—Common, February to March.

Symp.—Confined to stem, branches and peduncles (never seen on leaves). Infected parts hypertrophied and much curved. Growth snow-white in the form of long, cottony hanging threads or tufts.

[In association with *Cystopus candidus* (Pers.) (Lev.)]

Conidiophores.—Robust, emerging in clusters, upto 8 times dichotomously branched, 204–670 μ long ; trunk thick, erect, bulbous at base, 111–474 × 11–22 μ ; branches cover about one half the length of the trunk ; primary branches spreading, secondary ones curved, interlocking ; ultimate branchlets thick, sharp-pointed, equal, at acute angles, usually running parallel, often curved back and hook-like near the tip, upto 30 μ long.

Conidia.—Faintly greyish brown, globose to sub-globose without difference between apex and base, 15–22 × 11–18 μ.
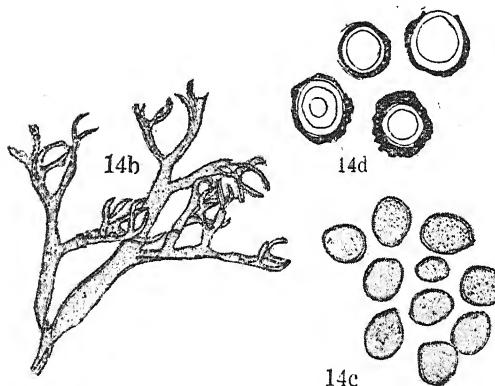
Fig. 14. *Peronospora parasitica* on *Eruca sativa*. Fig. *b*. A part of conidiophore (× 420). Fig. *c*. Conidia (× 280). Fig. *d*. Oospores (× 285).

Oospores.—Embedded in the host tissue, abundant, drab brown, 37–55 μ in diameter ; oogonial wall persistent, drab brown ; epispore irregular, deep brown, exine smooth, bright, greyish-white and completely spherical.

15. *Peronospora Sisymbryii-officinalis* Gäumann, *Beitr. Bot. Centr.*, XXXV, Abt. 1, p. 139, 1938 and *Beitr. Krypt. Sch.*, V, p. 276, 1923.

Host.—*Sisymbrium Irio* L.

Loc.—Common, February to March.

Symp.—Minute cottony tufts on brownish circular spots on the under-surface of the leaves, corresponding areas on upper surface pale, tufts white, scattered over entire area, rarely aggregated into small patches.

Conidiophores.—Drab brown, emerging through stomata in fascicles of 3 (2–4 or more), 3–8 times branched, 81–407 μ long ; trunk erect (never oblique), thick, drab brown, 19–259 × 11–15 μ ; branches covering about three-fourths the length of the trunk. Conidiophore branching rather peculiar ; trunk never bifurcates dichotomously at top but continues as such bearing branches on its right and left, trunk itself taking up the function of conidiophore growth ; gradually decreases in thickness as it grows ; no bend or curve and conidiophore looks erect with stretched out branches at the upper region. Lateral branches which are not borne dichotomously are the primary branches ; these branches subsequently divide dichotomously ; ultimate branchlets sharp-pointed like spines, curved, running into each other, acute angle, more or less equal, upto 25 μ long.
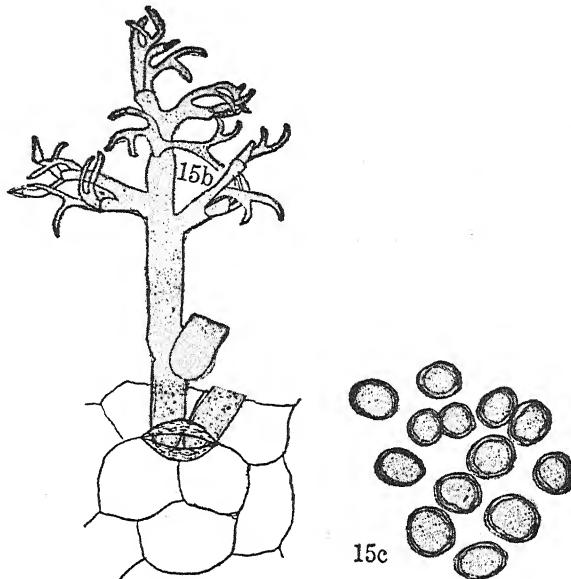
Fig. 15. *Peronospora Sisymbryii-officinalis* on *Sisymbrium Irio*. Fig. 15 *b*. Conidiophores coming out through a stoma (× 280). Fig. 15 *c*. Conida (× 280).

Conidia.—Sub-globose to broadly oblong, rounded at both ends, drab brown, wall blackish, 15–30 × 15–22 μ.

Oospores not observed.

Peronospora SPECIES ON Plantaginaceæ

16. *Peronospora plantaginis* Underwood, *Bull. Torey Bot. Club*, XXIV, p. 83, 1897.

Host.—*Plantago amplexicaulis* Cav.

Loc.—Lahore, March and April.

Symp.—Infected leaves become bleached on upper-surface, downy patches usually on the underside towards the apex or at margins which are curved inwards.

Conidiophores.—Solitary, 4–7 times dichotomously branched, erect, slightly robust, 67–592 μ long; trunk upto 448 × 6–11 μ; branches cover one-third of the length of the trunk and given off at acute angles; rachis straight and spreading; growth continued by the main central branch which appears to be the continuation of the trunk; ultimate branchlets small, curved, acute-angled, rarely at right angle, wide apart or running parallel, 5–15 μ long, thick, blunted, rarely sharp-pointed.
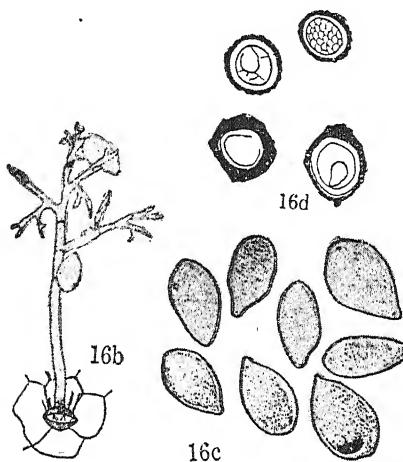
Fig. 16. *Peronospora plantaginis* on *Plantago amplexicaulis*. Fig. 16 *b*. Conidiophores coming out through a stoma (× 140). Fig. 16 *c*. Conidia (× 280). Fig. 16 *d*. Oospores (× 285).

Conidia.—Large, smoky (or dark brown), broader at one end and narrowed at the other with a papillum, ovoid-oblong or broadly ellipsoid, 33–46 × 15–30 μ.

Oospores.—In old and badly infected leaves; 22–45 μ in diameter; oogonial wall persistent and slightly brownish; epispore drab brown; rough, irregular; exine whitish grey, smooth, completely spherical.

[*Note.*—This species agrees most with Underwood's *P. plantaginis* on *Plantago aristata* but the presence of a papilla on the conidia and the oospores which were so far unknown raises a doubt regarding this identification. *Peronospora alta* of Fuckel on *Plantago lanceolata* L. and *P. major* L. has also oospores but their measurements given by Gäumann (1923), indicate that they are much smaller; the conidia of Fuckel's fungus are also smaller. A comparison of the Punjab fungus with the types of *Peronospora plantaginis* and *P. alta* can alone decide to which species this fungus belongs. The present determination is tentative.]

SUMMARY

In this paper 16 species of *Peronospora* occurring on 20 hosts in the Punjab have been described; six of these species are new records from this country and seven of the hosts are also new records. *Peronospora Brassicæ* Gäumann has been for the first time reported on *Malcolmia africana* Br.

ACKNOWLEDGMENTS

This work forms a part of a thesis submitted for the M.Sc. degree of the Panjab University and was carried out under the

supervision of Dr. H. Chaudhuri, Head of the University Department in Botany, to whom the author expresses his thanks for suggesting the problem and for guidance throughout the progress of the work. He is thankful to Dr. G. Watts Padwick for kindly permitting him to consult the literature and examine the *Peronospora* specimens in Mycology Section of the Imperial Agricultural Research Institute, New Delhi. He expresses his great indebtedness to Dr. B. B. Mundkur of the Imperial Agricultural Research Institute, with whom he discussed the question of taxonomy and nomen-. clature of the species of *Peronospora*, for the help in revising the manuscript and various other suggestions. In the end, he thanks the Vice-Chancellor of the Panjab University for kindly sanctioning him a small grant to meet his expenses for travelling to Delhi and spending a short time there for completion of the work.

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The localities in the Karewa beds of Kashmir mentioned in the report all lie within this circle.

AFGHANISTAN

N. W. Frontier Province
Samana Range
Salt Range
Pir Panjal Range

KASHMIR
Srinagar

TIBET

BALUCHISTAN

PUNJAB
Amritsar
Dehra Dun
Almora
Sukhidhanj

NEPAL

Nowgong District

BHUTAN

ASSAM

RAJPUTANA

UNITED PROVINCES

Lucknow
Benares
Mirzapur

SIND
Jhirak

CENTRAL INDIA

BEHAR
Jharia
Raniganj

BENGAL
Rajamahal Hills

Calcutta

BURMA

CUTCH
Kakadbhit
Bhuj

BOMBAY PRESIDENCY

DECCAN

Chhindwara
Mohgaon Kalan
CENTRAL PROVINCES

Nagpur

ORISSA

ARABIAN SEA

HYDERABAD STATE

MADRAS PRESIDENCY

BAY OF BENGAL

Middle Andaman
Long Island
ANDAMAN ISLANDS

MYSORE STATE
Bangalore

Trichinopoly

0   100   300
Miles

Tabbowa
Andigama
CEYLON

Ratnapura   Colombugama

We mourn the death of
ALBERT CHARLES SEWARD
(†11 April 1941)

Doyen of Palæobotanists
whose noble personality, no less than his vast
learning, was a fountainhead of inspiration to
the Indian school of palaeobotany.

PALÆOBOTANY IN INDIA

III

Progress Report for 1941

NOTE

THESE reports are published with the co-operation and authority of a working committee. They are based upon data for which the members are severally responsible, the convener acting as an editor.

Colleagues desiring to receive the reports should send a request to the convener, who will be glad to place their names on the mailing list.

We regret to say that owing to conditions arising out of the war the papers by C. Jacob, K. Jacob, A. R. Rao, B. Sahni and H. S. Rao, and R. V. Sitholey (announced in the report for 1940) of which the publication was expected in the *Memoirs of the Geological Survey of India* (Palæontologia Indica) cannot now be printed during the pendency of the war.

Department of Botany,   B. SAHNI,
 The University,   *Convener.*
 Lucknow, India,   R. V. SITHOLEY,
 February 17, 1942.   *Secretary.*

PERMO-CARBONIFEROUS

Australia.—D. D. Pant (Lucknow) is continuing his study of the microflora of some Australian tillites. In the Bacchus Marsh

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tillite from Victoria he has found, in addition to the kinds of spores previously recorded by Mrs. Jacob (Report for 1939, pp. 202–3), about a dozen types of unwinged spores, some spores with a single wing, two-winged spores including forms referable to *Pityosporites*, and three-lobed spores similar to *Brachytrilistrium* (Naumova). Two types of tracheids have also been obtained.

South Africa.—By macerating material of the Dwyka tillite from near Johannesburg sent by A. L. du Toit and T. N. Leslie, Pant has obtained some spores, bits of tissue with thick-walled cells, pieces of cuticle, and wood fragments.

Mirzapur District.—K. R. Mehta (Benares) is engaged at Lucknow in describing the microflora of some carbonaceous shales from the Singrauli coalfield, a part of which lies in the south-west corner of the Mirzapur district, U.P. The material was collected by R. C. Misra of Benares from a horizon in the Lower Gondwanas believed to be Barakar.

(*a*) *Winged spores*, 120 μ to 180 μ. Flat and disc-like with a central body surrounded by a frilled membranous wing which in some cases is radially striated. These spores recall the one-winged spores described by Mrs. Jacob from the Lower Gondwanas of India and Australia. Also a few spores with a roundly triangular body surrounded by a narrow wing, and some 2-winged spores of the *Pityosporites* type.

(*b*) *Unwinged spores*.—Three megaspores (319–388 μ) with triradiate mark and warty or granular surface. Round microspores (66 μ to 96 μ) with triradiate mark; also some clumps of much smaller (24–44 μ) thin walled microspores without the trilete; and some roundly triangular microspores with at least three different types of surface features.

(*c*) *Cuticles*.—Several types of cuticles bearing stomata surrounded by 4 to 6 or 7 subsidiary cells. Some have papillate epidermal cells. The papillæ on the subsidiary cells are well preserved and seem to provide a sort of interlocking arrangement for the protection of the stomatal openings. These papillate cuticles bear a close resemblance to those of *Lebachia* (R. Florin, Palæontographica, ' Die Coniferen des Obercarbons und des unteren Perms ', 1–4, 1938–39).

(*d*) *Stone cells* of various shapes and sizes and clear canals traversing the thickening layers right down to the middle lamella.

(*e*) *Woods and tracheids*.—About two dozen types of wood in the form of well preserved shreds. Some of the tracheids show spiral thickening bands combined with bordered pits (Taxinean sculpturing).

Lower Gondwana coals. K. Jacob and Mrs. Jacob (Calcutta) are investigating the microflora of Gondwana coal seams and have obtained spores from the Satpukuria and Ghusik seams in the Raniganj Coalfield, from the Top Seam at Angul, and from certain seams in the Jharia coalfield.

TRIASSIC

Salt Range.—R. V. Sitholey (Lucknow) has now completed the description of the fossils collected by E. R. Gee. The microsporophyll bearing pendulous sporangia in epaulette-like clusters has been referred to *Indotheca sakesarensis* n.g. et sp. This is the first record of a pteridospermous microsporophyll from India. The paper, entitled *Plant remains from the Triassic of the Salt Range in the Punjab*, will be published by the Geological Survey of India.

JURASSIC

Rajmahal Hills.—A. R. Rao (Lucknow) is continuing his work on the silicified flora of the Rajmahal hills, Behar. Two new species of *Pityosporites* and two of three-winged spores (possibly Podocarpinean) have been discovered, besides some sporangia and spores of uncertain affinity.

Salt Range.—B. Sahni and R. V. Sitholey (Lucknow) have continued work on the specimens collected by E. R. Gee. Two new species of *Phlebopteris* (*P. hirsuta* and *P. indica*) with well preserved sporangia and spores are created. Three sterile ferns are provisionally described as species of *Cladophlebis* (? *Phlebopteris*). The cycadophyta include three new species of *Otozamites*, one of which has been named *Otozamites pecten* sp. n., and a few badly preserved leaves of *Ptilophyllum*. Among the conifers three distinct (probably new) species of *Brachyphyllum* are recognisable.

Afghanistan.—K. Jacob is describing a collection of well preserved fossils brought by W. D. West from the Saighan series. Several species are recorded for the first time from this area. Well preserved spores of *Klukia exilis*, as well as cuticles of *Ctenis* sp. and *Pterophyllum* sp., have been obtained by maceration.

Andigama (Ceylon).—Miss Janet's unfinished work on the microflora of the Andigama shale is being completed in a joint paper with Sahni who has macerated a quantity of further material from the same locality. Among the 18 or 19 different types of cuticles so far recovered a considerable proportion shows deeply sinuous epidermal cell walls and transversely placed stomata of the syndetocheil type, indicating that the Bennettitales were strongly represented in the flora. Others have haplocheil stomata suggesting a Cycadean affinity. One of the ten or more species of woods has tracheids with a mixed pitting suggestive of either a homoxylous angiosperm or a cycadeoid ; there are no true vessels. The rest of the woods are of coniferous types. The composition of this microflora is strongly in favour of assigning the Andigama shale to a Jurassic horizon, and therefore confirms the opinion of Deraniyagala tentatively[1] expressed on other grounds. Some of

[1] 1939. A carbonaceous Jurassic shale from Ceylon. *Ceylon Journ. Sci.*, Sect. B, Pt. 3, p. 193.
1940. Gondwana deposits of Ceylon. *Proc. 27th Ind. Sci. Congr.*, Pt. IV, p. 77.
1941. Some botanical fossils from Ceylon. *Journ. Roy. As. Soc., Ceyl. Branch*, Vol. 35, No. 93, p. 57.

these cuticles may belong to Upper Gondwana species of Cycado-phyta already known from leaf impressions in the Madras coast outliers.

A peculiar flat seed-like body with a dark nucule and a thin border is of unknown affinity. Considering the large proportion of Bennettitales in this flora it may be possible to refer at least some of the microspores, and perhaps also this " seed", to that group. Maceration of further material promises useful additions to our knowledge of the Upper Gondwana flora of Ceylon.

Ceylon (Tabbowa and Andigama).—R. V. Sitholey has completed a paper entitled *Jurassic plants from the Tabbowa series in Ceylon.* The material was sent by Mr. D. N. Wadia, Mineralogist to the Government of Ceylon; it includes about twelve different plant types of which two, *viz., Cladophlebis* sp., and *Elatocladus plana* come from Andigama; the rest were collected from Tabbowa. The genera *Ptilophyllum* and *Otozamites* are recorded for the first time from the island. Two new species, *Cladophlebis zeylanica* and *Sphenopteris Wadiai,* are created.

JURASSIC OR CRETACEOUS

Trichinopoly district.—K. Jacob and N. K. N. Aiyyengar (Calcutta) are describing from Varagur, near Ariyalur in the Trichi-nopoly district, a specimen provisionally referred to *Cycadeoidea.* The fossil (discovered by Aiyyengar as a loose block on the surface of the ground) is badly preserved, and no flowers are available. But wedged in between the large rhomboid foliage-leaf scars there are several groups of smaller scars arranged like the bracts round the floral peduncles of a *Cycadeoidea.* The locality lies further south than the present southernmost limit of this genus. Although not found *in situ* the fossil is probably of Cretaceous age : it was picked up from an area mapped as Cretaceous.

LOWER CRETACEOUS

Cutch.—T. S. Mahabalé (Ahmedabad) has made a collection of Upper Gondwana plants including ferns, conifers, and cycado-phyta as well as some seed-bearing scales and petrified wood from Bhuj and Kakadbhit (Cucurbit) in Cutch. On maceration some of the specimens have yielded spores and tracheids.

TERTIARY

Assam (Eocene).—K. S. Rao (Tumkur, Mysore State) has com-pleted the investigation of the limestone algal flora. The plants belong to two distinct families : Corallinaceæ and Dasycladaceæ. A paper entitled *Fossil Algæ from Assam,* I : *The Corallinaceæ* has been sent for publication in the *Records of the Geological Survey of India.* The paper describes two new species of *Archeolitho-thamnium, A. nongsteinensis* and *A. langrinensis.* The occurrence of these forms in the limestones of this area serves to fix the age of these doubtfully Cretaceous beds as Eocene. The evidence of the

algæ is not at variance with the recent work of C. S. Fox and A. M. N. Ghose proving that the Cherra sandstones belong to the Lower Tertiary age.

The report on the Assam Dasycladaceæ is now being prepared as a separate paper for the press.

Deccan Intertrappean.—B. Sahni has published the following paper: "*Indian silicified plants*—1. *Azolla intertrappea Sahni and H. S. Rao,*" *Proc. Ind. Acad. Sci. Sec. B.,* Vol. XIV, 1941, pp. 489–501, with three plates and one map. A megasporocarp is figured with massulæ anchored to its filaments by means of the glochidia.

Chhindwara.—V. B. Shukla (Lucknow) has described a second new species of *Dadoxylon* stated to have been collected from near Chhindwara. Like *Dadoxylon Deccani* Shukla, to which this new species is related, it shows a combination of alternate and opposite pits. He has also described a gymnospermous wood, *Cupressinoxylon intertrappeum* sp. nov., with the bordered pits occasionally alternate. In this feature this wood resembles *C. alternans* Sahni in which alternate pitting is an almost constant feature.

Mohgaon Kalan.—Shukla collected on 8th March and subsequent dates about 35 specimens of petrified angiospermic flowers from Mohgaonkalan. The preservation of most of the flowers is so perfect that it has been possible to make a complete floral diagram and floral formula. The flowers average 6 mm. in length and are stalked. They possess a calyx, a gamopetalous 8-lobed tubular corolla, 8 epipetalous stamens and a shortly stalked superior 8-locular ovary with axile placentation. The anthers are seen in different stages of dehiscence; they all contain pollen-grains. A few pollen grains have been found lying in the corolla tube. The flowers were definitely heterostylous; most of them are short-styled and the rest long-styled. The stigma is capitate. Several vascular bundles have been found in the wall of the ovary leading up to the stigma. The floral structure helps to identify the plant as a member of the Lythraceæ. The characters of the ovary show such a close resemblance with the fruit *Enigmocarpon Parijai*, described by Sahni from the same locality, that there is hardly any doubt about the fruit and the flower belonging to the same plant. Both the flowers and the fruits are abundant at this locality and frequently occur in close association with each other; neither of them have been found at any other locality. For the flower the new generic name *Sahnianthus* is proposed.

Shukla has also described a noded monocot stem (2·3 cm. in diameter) from the same locality. The vascular bundles appear to be without any fibrous part and the wood seems to resemble that of some solid bamboos.

Almora district.—B. Sen (Almora) has found some leaf impressions of dicotyledons on boulders lying on the surface near the Vivekananda Ashram at Shyamalatal (Sukhidhanj P.O.) not far from the junction between the Sarda and Ladhiya rivers at the western border of Nepal.

Bihar.—Of the 27 wood specimens under investigation by K. A. Chowdhury (Dehra Dun) one shows similarity to *Dipterocarpo-xylon garoense* Chowdhury. This specimen comes from the laterite deposits of the Raniganj coalfield and its identification is of interest as some doubt exists about the correct age of the deposits.

Assam.—From an investigation of the wood specimens collected from the bed of Tailangthu river K. A. Chowdhury and a co-worker have found that one specimen shows a great resemblance to the living genus *Kayea* (Guttiferæ). Out of twenty other specimens collected from near Lumding, Nowgong, 15 have been identified by Chowdhury as *Glutoxylon.*

Burma.—One of the woods from Burma investigated by Chowdhury exhibits some resemblance with *Terminalia.*

North West Frontier Province.—S. R. N. Rao (Bangalore) has published the following paper: "*An algal flora from the Lockhart Limestone (Ranikot Series) of the Samana range, North Western India,*" *Journal of the Mysore University*, Vol. 11, pp. 41–53, 1941, with two plates. To the new species previously reported have now been added *Merophyllum daviesi* sp. nov. and *Neomeris (Larvaria* ?) sp.

Sind.—S. R. N. Rao in "*A preliminary report of some calcareous algæ from the uppermost Ranikot bed of Jhirak, Sind,*" records the genera *Neomeris (Larvaria), Dactylophora* ? and *Lithothamnium.*

Andaman Islands.—S. R. N. Rao has completed the investigation of the algal flora from Long Island in the Middle Andamans. His paper entitled "*Calcareous algæ of the sub-family Corallineæ from a* Lepidocyclina—*limestone from the Andaman Islands*" describes *Amphiroa oceanica* sp. nov. and *Corallina andamanensis* sp. nov. The latter has been shown to be different from *Corallinia grandis* (= *Lithothamnium grandis* Das Gupta). The age of the Andaman flora on the evidence of the associated foraminifera is considered to be late Oligocene or early Miocene.

PLEISTOCENE

G. S. Puri (Lucknow) is continuing the description of the Karewa flora to which considerable additions have been made in recent months. All collections (about 21 in number) so far made from these beds have been acquired and detailed studies are in progress on this material, which now comprises several thousand specimens gathered by de Terra, B. Sahni, R. R. Stewart, T. R. Chadha, P. Bhasin, Ram Nath, S. S. Gergon, R. Sarup and the author, besides the earlier collections by Middlemiss. The localities are Laredura (6,000 to 6,500 ft.), Dangarpur (6,300 ft.), Naugam (6,300 ft.), Nagbal (6,500 ft.), Satar Siran (about 6,500 ft.), Nilasar (about 6,400 ft.), Nambil Nar (7,000 ft.), Hajabal (about 7,500 ft.), Tsunt Pathri (7,200 ft.), Gogajipathri (8,800 ft.), Liddarmarg (10,600 ft.), Botapathri (9,500 ft.), and Ningal Nullah (9,000 to 9,800 ft.).

The fossiliferous outcrops at Laredura and Liddarmarg were first thought by De Terra (see R. P. Wodehouse and H. de Terra, 1935, "The Pleistocene pollen of Kashmir," *Mem. Conn. Acad.*, Vol. IX, pp. 1 and 5) to be a part of the Upper Karewa formations but now he considers the fossiliferous beds at all the above-mentioned localities to belong to the upper part of the Lower Karewas (H. de Terra and T. T. Paterson, 1939, "Studies on the Ice Age in India and associated human cultures," *Carneg. Inst. Washington*, pp. 109–14, and Pl. LV).

The following further genera (with the number of species given in brackets) are described from the new material:—*Ranunculus* (1), *Clematis* (1), *Ceratophyllum* (1), *Nelumbium* (1), *Myriophyllum* (1), *Prunus* (2), *Rosa* (2), *Rubus* (3), *Indigofera* (2), *Salix* (6), *Populus* (4), *Betula* (3), *Carpinus* (2), *Corylus* (1), *Celtis* (1), *Elaeodendron* (1), *Sageretia* (1), *Leea* (1), *Aesculus* (1), *Rhus* (4), *Lannea* (1), *Juglans* (1), *Engelhardtia* (1), *Marlea* (1), *Hedera* (1), *Fraxinus* (2), *Olea* (1), *Hamiltonia* (1), *Randia* (1), *Wendlandia* (1), *Viburnum* (2), *Lonicera* (1), *Leycesteria* (1), *Inula* (1), ? *Sparganium* (1), *Picea* (1), *Cedrus* (1).

In addition to these some more species of the following genera, which were reported last year (Report for 1940, p. 8) have also been recognised from the new material:—*Berberis* (2), *Trapa* (1), *Pyrus* (4), *Cotoneaster* (3), *Spiraea* (1), *Desmodium* (3), *Quercus* (3), *Ulmus* (3), *Rhamnus* (1), *Acer* (6), *Cornus* (1) and *Myrsine* (1).

The Karewa beds have so far yielded 122 species belonging to 62 genera and 34 families of angiosperms and 6 species belonging to 5 genera of gymnosperms, besides some ferns. The families best represented are Salicaceæ with 10 species, Papilionatæ with 9, Acraceæ with 8, Fagaceæ, Betulaceæ, and Lauraceæ with 5 each, Anacardiaceæ, Caprifoliaceæ, Oleaceæ and Rhamnaceæ with 4 species each.

While the flora as a whole is modern in its composition it includes a larger number of species which do not grow in the Kashmir valley to-day. The five species of *Quercus*, which are very abundant in the fossil beds, are not to be seen in the present vegetation of the Pir Panjal Range or in the Kashmir valley. This suggests that physical and climatic conditions have considerably changed since the time when the valley was occupied by oak forests. Another interesting fact is the difference between the altitude at which the fossil species have been discovered and the height at which their modern representatives now grow in the North-West Himalayas. Some species of water plants, *e.g.*, *Trapa*, have been found in very great abundance in the fossil state at a considerably higher altitude (9,500 ft. or even more) than the level at which they are seen growing to-day (5,200 ft.) in the valley. There thus appears to be evidence of uplift by at least 5,000 ft. of the fossil beds, since the time they were deposited (see B. Sahni, 1936, "The Karewas of Kashmir," *Current Science*, Vol. V, p. 11).

Ceylon (Ratnapura district). G. S. Puri's description of the fossil stem sent by P.E.P. Deriyaniyagala has been published in a

paper entitled " *A fossil bamboo stem and some associated plant remains from the gem deposits of Ratnapura district, Ceylon,*" *Spolia Zeylanica,* Vol. XXIII, Pt. I, pp. 23–26, 1941, 3 Pls., 2 Text-figs. The stem has been identified as *Bambusa vulgaris,* Schrad: on maceration the wood was found to be heavily infected by a fungus with septate and branched hyphæ. The leaf impressions found in the pith-cast have been identified with *Wrightia flavido-rosea* Trim. Both these plants are represented in the modern flora of Ceylon and their occurrence in the gem deposits confirms Deriniyagala's view regarding the Upper Pleistocene age of the beds.

LABORATORY TECHNIQUE

In a brief note " *Permanent labels for microscope slides,*" *Current Science,* Vol. X, No. 11, November 1941, pp. 485–86, Sahni suggests that fossil slides can be effectively labelled by writing with a lead pencil on the ground surface of the slide and then putting on a coverslip. The Canada balsam makes the ground surface transparent again, leaving the pencil writing on a clear background. Such labels are particularly suitable for damp tropical climates.

NEW MEMBERS

K. R. MEHTA, ESQ., M.Sc., Assistant Professor of Botany, Benares Hindu University, *Benares City.*

D. D. PANT, ESQ., Research Fellow, Botany Department, University of Lucknow, *Lucknow.*

NEW ADDRESS

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TRIPLASTRUM, A NEW MEMBER OF THE DESMIDIACEÆ FROM SOUTH INDIA*

By M. O. P. IYENGAR, M.A., PH.D. (LOND.), F.L.S.

AND

K. R. RAMANATHAN, B.SC. (HONS.), M.SC.

THE alga forming the subject of this communication was collected in December 1940 from a paddy field near Madras. It occurred in a stray manner among other algæ and was present only for a very short period.

The alga is small, straight and cylindrical with a well-defined median constriction (Text-figs. 1–3; Pl. IX, Fig. 1). It is 13–14 μ broad and is about 8–10 times as long as broad. The ends of the semi-cells are somewhat dilated and trilobed, each lobe being broadly rounded and bearing two to four short spines (Text-figs. 1–5; Pl. IX, Fig. 1). The cell wall is smooth and hyaline. In each cell there are four chloroplasts arranged in a median row, each semi-cell having two chloroplasts (Text-fig. 1; Pl. IX, Fig. 1). The chloroplasts are axile with 6–8 radiating plates and a large central pyrenoid (Text-fig. 1; Pl. IX, Fig. 1). The nucleus is situated at the isthmus region.

The zygote is formed in the usual manner by two cells coming near each other and becoming enveloped in a common mucilage (Text-fig. 3). It is spherical and has a thick wall with a crenate margin and measures 42·0 μ in diameter (Text-fig. 3; Pl. IX, Figs. 2, 3).

DISCUSSION

The alga in its elongated shape, circular cross-section and in having two median axile chloroplasts with radiating plates in each semi-cell, comes very close to *Penium*. But it differs from *Penium* in having trilobed terminal portions. It resembles *Pleurotænium* to a certain extent in its general shape, but is quite distinct from *Pleurotænium* in having trilobed ends and also in having two median axile chloroplasts in each semi-cell. Again, it shows some resemblance to *Icthyocercus*, but in *Icthyocercus*, the ends are flattened and bilobed as in *Tetmemorus*, whereas in the present alga the ends are definitely trilobed. Finally, in having trilobed ends, it shows some resemblance to *Staurastrum*, but it differs from *Staurastrum* in having two median axile chloroplasts in each semi-cell.

From the foregoing it is quite evident that the alga combines in a way some features or other characteristics of each of the above-mentioned genera, but cannot be referred to any one of them. It

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* From the Department of Botany, University of Madras.

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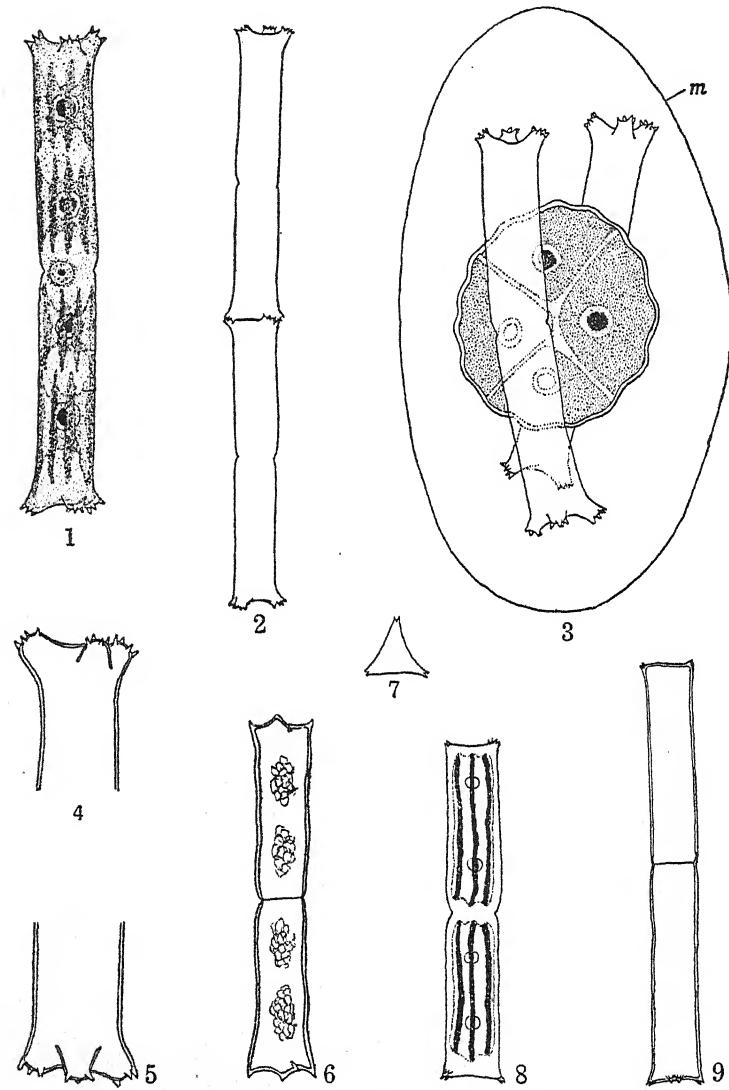
Text-figs. 1–9.—Fig. 1. A cell showing the chloroplasts, nucleus and the trilobed ends ; each lobe bearing 3 spines (× 725). Fig. 2. Two cells just after division remaining in contact with one another (× 550). Fig. 3. Two cells conjugating with one another with the fully formed zygote in between them (× 550). (Drawn from living material) (*m*, limit of the mucilaginous envelope). Figs. 4 and 5. The ends of two different specimens to show quadri-spinate and trispinate lobes (× 1125). Fig. 6. *Triplastrum abbreviatum* (Turner) comb. nov. (Redrawn from Turner, 1892) (× 750). Figs. 7–9. *Triplastrum simplex* (Allorge) comb. nov. (Redrawn from Allorge, 1924) (× 715).

appears, therefore, best to keep it in a new genus which may be called *Triplastrum* gen. nov. The alga may be named *Triplastrum indicum* gen. et sp. nov.

A desmid somewhat very closely resembling the present alga was described by Turner (1892) and referred by him to the genus *Triploceros* under the name of *Triploceros abbreviatum* Turner (Text-fig. 6). The description of this desmid is as follows : " Frond linear, inflated at extremities ; showing a constriction at the central portion which forms a shallow angular notch on each side. Ends truncated and inflated, the inflated portion forming 3–4 shallow lobes, each terminated by a short tooth. A very rare species of which I have obtained only two specimens. G. C. Wallich " (Turner, 1892, p. 27).

According to Bailey who established the genus *Triploceros*, the main characteristics of the genus are : Frond binate ; segments straight, much elongated, with numerous whorls of knot-like projections ; ends of the segments three lobed ; lobes bidentate " (Bailey, 1850, p. 37). Thus the two main features of the genus as originally defined are, (1) the whorls of knot-like projections and (2) the three-lobed ends of the segments. Turner (1892, p. 27), while referring his desmid to the genus *Triploceros*, states : " This little form seems a trifle anomalous, when compared with others of the genus, but I think the terminal lobes decide its position." This means that Turner completely ignores the first main feature of the genus *Triploceros*, *viz.*, the whorls of knot-like projections, and takes into consideration only the second feature of the genus, *viz.*, the lobed ends of the segments. This ignoring by Turner of one of the two main features of the genus, *viz.*, the whorls of knot-like projections, in order to include his alga in the genus *Triploceros* is, in the opinion of the present authors, quite unwarranted. Moreover, even the terminal lobes of *Triploceros abbreviatum* as figured by Turner are not like those of a *Triploceros* at all (*cf.*, West, 1911, pp. 55–56, Fig. 7; Smith, 1924, Pl. 55, Figs. 5–9; Krieger, 193, Pl. VIII, Figs. 3, 3 *a*). Again according to West who examined specimens of *T. gracile* which were collected by him from the same area as the one from which Bailey collected his form, the terminal portions of the segments are flat and bear "two divergent processes somewhat obliquely disposed and bispinate (rarely trispinate) at their extremities. Alternating with these processes are two shorter apical lobes each of which terminates in an upwardly curved spine " (West, 1911, p. 56). Thus both in the absence of the whorls of the verrucæ and in the nature of the terminal lobes, Turner's desmid is quite unlike a *Triploceros* and so, in the opinion of the authors, will have to be removed from that genus.

On the other hand, Turner's alga, in its general shape and structure, shows a great resemblance to the present desmid and may, therefore, be included in the present new genus *Triplastrum* and named as *Triplastrum abbreviatum* (Turner) comb. nov. The chloroplast of Turner's form has not been described by him, but,

judging from his figure (Turner, 1892, Pl. IV, Fig. 17), the chloro-
plasts are evidently four, two in each semi-cell, and placed in a
median row as in the present alga.

Allorge (1924, p. 464) described from France a desmid which
resembled *T. abbreviatum* Turner and followed Turner in referring
this desmid to the genus *Triploceros* as *Triploceros simplex* (Text-
figs. 7–9). This alga also will have to be removed from the genus
Triploceros for the reasons stated already in the case of *T. abbre-
viatum* and included in the present genus as *Triplastrum simplex*
(Allorge) comb. nov. In this form, however, Allorge describes the
chloroplast as single in each semi-cell. But his figure (Text-fig. 8)
shows two medianly placed pyrenoids in each chloroplast suggesting
that what appears as a single chloroplast is very probably double
as in the present alga.

DESCRIPTION

Triplastrum gen. nov.

Cells small, elongate, cylindrical with a well-defined median
constriction; semi-cells straight, with nearly parallel sides; ends
three or four lobed, each lobe bearing one or more short spines;
chloroplasts four, two in each semi-cell; arranged in a median row
each chloroplast axile with radiating plates and a central pyrenoid;
zygospore spherical, thick-walled with a crenate margin.

Triplastrum indicum sp. nov.

Cells small, 8–10 times as long as broad, with a well-defined
median constriction; semi-cells straight, cylindrical, with sides
nearly parallel; ends slightly inflated, trilobed, each lobe broadly
rounded and bearing 2–4 short spines; cell wall smooth and hyaline;
chloroplasts two in each semi-cell, arranged in a median row; each
chloroplast axile, with radiating plates and a central pyrenoid;
zygospore spherical, thick-walled with crenate margin; cells $80 \cdot 5$–
$91 \cdot 9 \mu$ long; $13 \cdot 4$–$14 \cdot 0 \mu$ broad at the base of the semi-cells and
$14 \cdot 0$–$16 \cdot 7 \mu$ broad at the apices; isthmus $11 \cdot 7 \mu$ broad; zygote
$42 \cdot 0 \mu$ in diameter.

Habitat.—Among other algæ in a paddyfield, Madras, South
India.

Triplastrum abbreviatum (Turner) comb. nov.

(*Triploceros abbreviatum* Turner. " The Freshwater Algæ of
India," *Kungl. Svensk. Vetenskap. Akad. Handl.*, 1892, **25**, p. 27.)

Cells small about 6 times as long as broad with a shallow median
constriction; semi-cells straight, cylindrical with nearly parallel
sides; ends truncated and inflated, the inflated portion forming
3–4 shallow lobes, each lobe bearing a single spine; membrane
smooth; chloroplasts probably two in each semi-cell arranged in a
median row; zygospore not known; cells 65–85 μ long; 11–15 μ
broad at the isthmus; 13–17 μ broad at the apices.

Habitat.—Raneegunge, North India (Wallich).
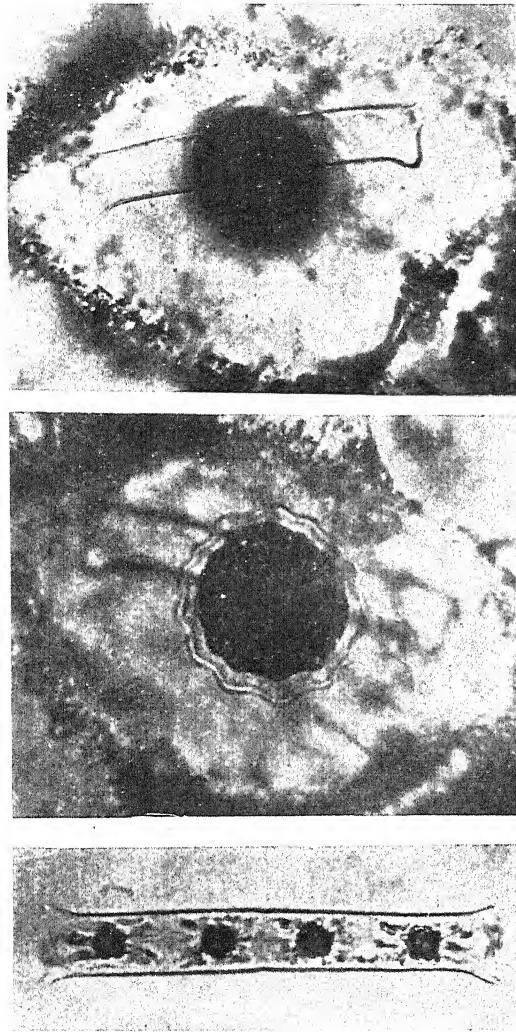
FIG. 1. Photomicrographs of a cell showing the four axile chloroplasts and trilobed ends. each lobe bearing three short spines (× 700).

FIG. 2. Photomicrograph of two conjugating cells with the zygote lying between them (× 520).

FIG. 3. Same as Fig. 2 taken at another focus to show the empty cells (× 520).

M. O. P. IYENGAR AND K. R. RAMANATHAN—*TRIPLASTRUM INDICUM* GEN. ET. SP. NOV.
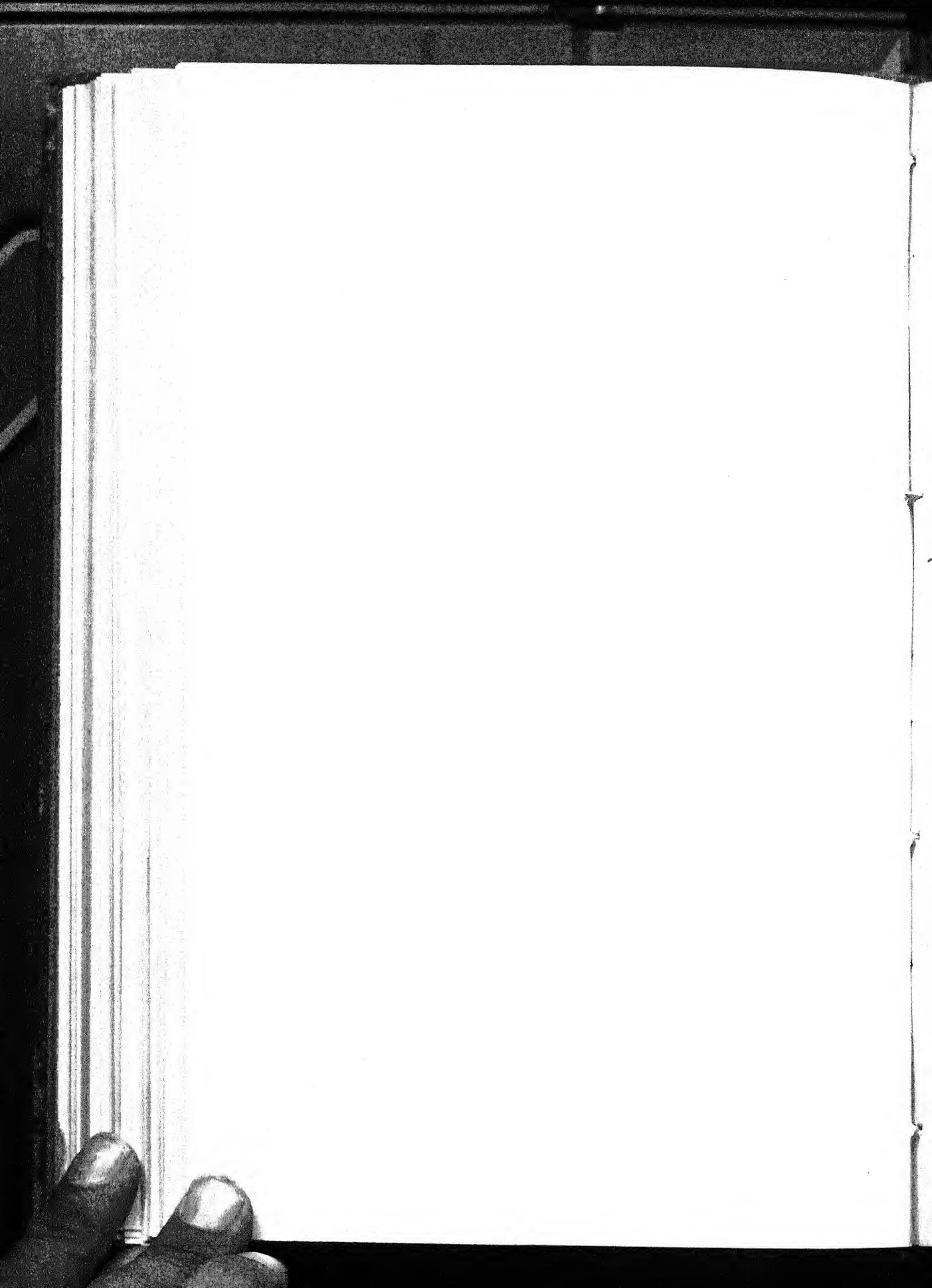
Triplastrum simplex (Allorge) comb. nov.

(*Triploceros simplex*, Allorge. " Desmidiees du Lac de Grand-Lieu," *Rev. Algol.*, 1924, **1**, p. 464.)

Cells small 6–10 times as long as broad with a shallow median constriction ; semi-cells straight, cylindrical with nearly parallel sides ; ends not prominently inflated, trilobed, each lobe bearing two spines ; membrane smooth and hyaline ; chloroplast one (two ?) in each semi-cell ; each chloroplast axile with 4–5 radiating plates ; zygospore not known ; cells 60–75 μ long ; 7·5–9·0 μ broad ; 9–10·5 μ broad at the apices (without spines) and 10–11·5 μ broad (with spines) ; isthmus 7–8 μ broad.

Habitat.—In lake Grand-Lieu, Paris, France (Allorge).

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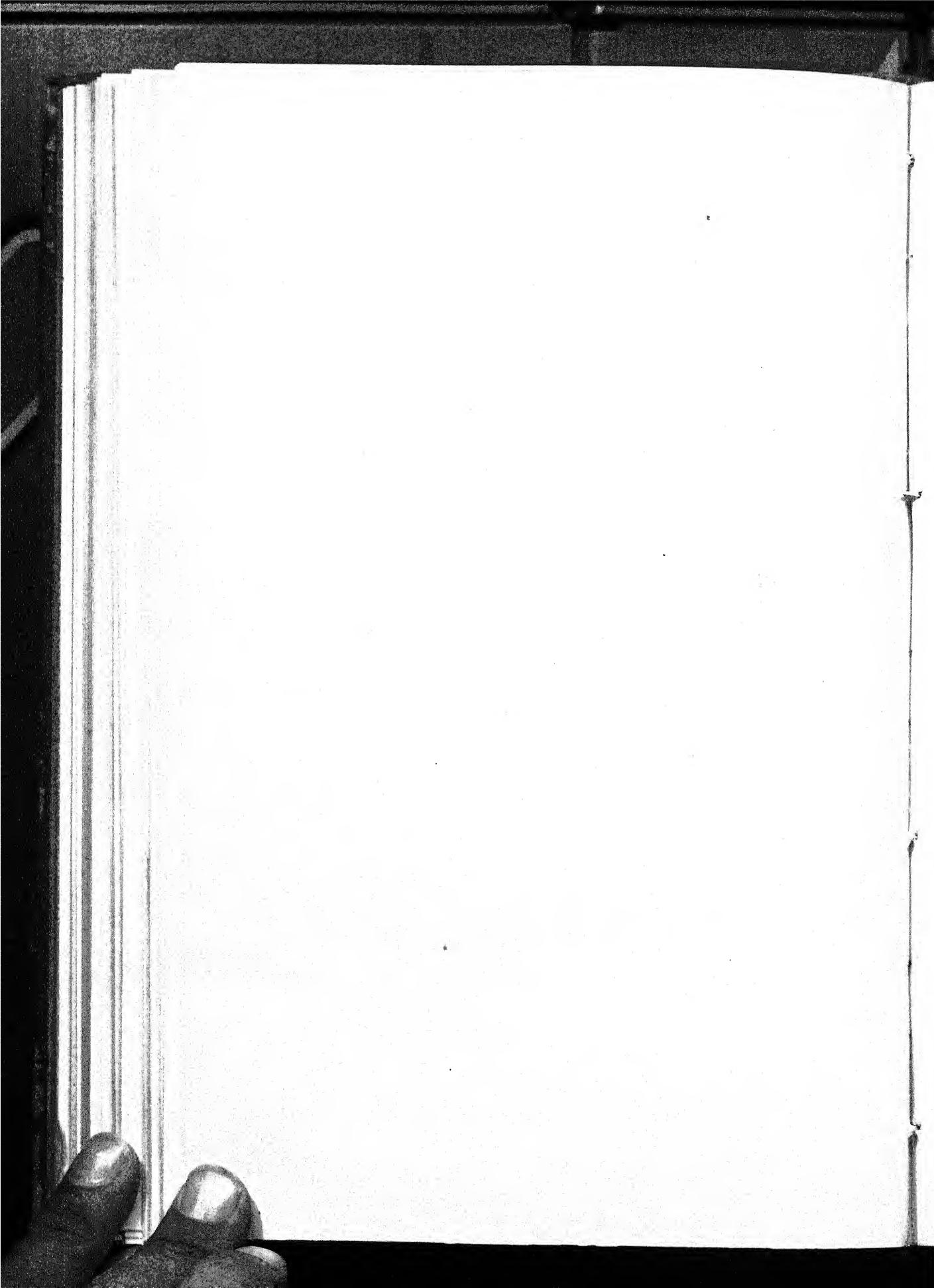
ON REDUCTION DIVISION AND AUXOSPORE-FORMATION IN *CYCLOTELLA MENEGHINIANA* KÜTZ.*

(PRELIMINARY NOTE)

BY M. O. P. IYENGAR, M.A., PH.D. (LOND.), F.L.S.

AND

R. SUBRAHMANYAN, B.SC.

IT is now fairly well established that auxospore-formation in the Pennales is the result of a sexual process. In the Centrales, on the other hand, auxospore-formation is considered to be an asexual process and not a sexual one. Again, the vegetative phase in the Pennales is considered to be diploid, reduction division taking place during auxospore-formation, whereas, in the case of the Centrales, the vegetative phase is generally held to be haploid (Hustedt, 1930, p. 9). But, some of the recent investigations on the group tend to point out that auxospore-formation in the Centrales also is brought about by a sexual process as in the Pennales and that the vegetative phase even in the Centrales is diploid as in the Pennales, reduction division taking place during auxospore-formation.

Persidsky (1929) found in two species of *Chætoceros* that before auxospore-formation the nucleus divides twice and forms four nuclei. The first division, according to him, is meiotic. He presumes that two of these four nuclei fuse and the remaining two degenerate.

Cholnoky (1933) found in the young auxospores of *Melosira arenaria* one large nucleus and two small degenerating ones. The large one becomes the nucleus of the auxospore. He presumes that reduction division takes place before auxospore-formation and that, out of the four nuclei formed, two fuse and form the large nucleus of the auxospore and the other two degenerate.

Geitler (1934, p. 423) mentions that he found in the auxospores of an undetermined species of *Melosira* one functioning nucleus and one or two degenerating ones.

In 1935, Persidsky found that during auxospore-formation in *Melosira varians* four nuclei are formed by two successive divisions, of which the first is a reduction division. Two of these four nuclei fuse and form the nucleus of the auxospore, while the remaining

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9

two degenerate. Thus, the fusion of the two nuclei which he presumed in the case of *Chœtoceros* (Persidsky, 1929) was proved by him to be an actual fact in *Melosira varians*.

The above-mentioned investigations suggest (1) that auxospore-formation in these members of the Centrales (*Chœtoceros* spp., *Melosira arenaria* and *M. varians*) is the result of a sexual process as in the Pennales (through the autogamous fusion of two gametic nuclei) and (2) that the vegetative cells are diploid as in the Pennales and undergo reduction division during auxospore-formation. But algologists, while inclined to accept in a way the above conclusions, still appear to feel that the case for the Centrales is not quite fully established. Fritsch (1935, p. 620) commenting on the observations of Persidsky (1929) on *Chœtoceros* and of Cholnoky (1933) on *Melosira arenaria* states : " The possibility of a reduction division and of subsequent autogamy cannot be denied, but further research will be necessary to substantiate this clearly." Again, later on, after reviewing the evidence for reduction division in Centric diatoms, Fritsch (1935, p. 637) states : " Whatever may ultimately prove to be the correct reading of all these facts, it now seems clear that the Centrales are diploid like the Pennales, and the view that the former were haploid, which held sway for some little time on a rather inadequate basis, may be regarded as of historical interest only." Geitler (1935, p. 160) states : " Sexual reproduction among the Centrales, with the exception of *Melosira*, is not fully understood. It is probable, however, that the Centrales are also diplonts." Smith (1938, p. 213) when dealing with the Centrales refers to all the investigations mentioned above and finally states : " The nuclear behaviour in the foregoing cases is not established beyond all doubt, but there is a presumption that auxospore-formation is sexual in nature since it involves a fusion of two haploid nuclei. There is also a possibility that auxospores of other Centrales are formed in a similar manner. If this be true, vegetative cells of Centrales are diploid instead of haploid."

Since 1935, two more records of auxospore-formation in the Centrales have been made. F. Gross (1937–38) found in the auxospores of *Ditylum Brightwelli* (West) Grun. one large nucleus and two smaller ones and interprets his observations in the same way as Cholnoky (1933) did his *Melosira arenaria*. Reith (1940) found during auxospore-formation in *Melosira arenaria* a large nucleus and a degenerating residual nucleus and states that his observations correspond with those of Cholnoky (1933) on the same diatom.

Thus so far five members of the Centrales, *viz.*, *Chœtoceros* 2 spp. (Persidsky, 1929), *Melosira arenaria* (Cholnoky, 1933 and Reith, 1940), *Melosira* sp. (Geitler, 1934), *Melosira varians* (Persidsky, 1935) and *Ditylum Brightwelli* (Gross, 1937–38), have been investigated. Of these, only in *Melosira varians*, as Geitler (1935, p. 160) rightly points out, are the details of sexual reproduction

and alternation of nuclear phases known with a certain amount of completeness.

The authors found auxospore-formation taking place in their cultures of another member of the Centrales, *viz., Cyclotella Meneghiniana* Kütz. collected from the Parthasarathy Temple tank, in Triplicane, Madras. They made a detailed study of the life-history of the diatom with special reference to the nuclear changes taking place during auxospore-formation. Their observations are given briefly here below.

Plenty of cell-division was taking place throughout in the cultures and the diatom remained for a long time in a vegetative condition. After the individuals, as a result of repeated cell-division, have become very small in size, auxospore-formation is noticed in the cultures. The nucleus of the cell which is to give rise to an auxospore divides twice and forms four nuclei. The first division is heterotypic. During the prophase of this first division the nucleus enlarges in size and the fine reticulate structure characteristic of the resting nucleus disappears. Soon, long chromosomal threads become evident in the nucleus. The chromosomes then gradually become thicker and are finally seen lying on one side of the nuclear cavity (synizesis) (Pl. X, Fig. 1; Text-fig. 1). The paired nature of the threads can be made out on very careful examination. In the next stage (pachytene) the threads are seen distributed more uniformly in the nucleus and are thicker and shorter than in the previous stage (Pl. X, Fig. 2; Text-fig. 2). The threads then become still shorter and exhibit their paired nature very clearly. The next stage observed in two preparations was diakinesis. During this stage the bivalents are seen distributed near the periphery of the nucleus (Pl. X, Figs. 3 and 4; Text-figs. 3–5). The number of bivalents appears to be about 32–34 (n). The nuclear membrane and nucleolus disappear soon after diakinesis. In metaphase the bivalents are seen very compactly arranged more or less in a ring at the equator of the spindle. After anaphase and telophase two daughter nuclei are organised (Text-fig. 6).

The two nuclei next undergo the homeotypic division (Text-fig. 7) and four nuclei are formed (Pl. X, Fig. 5; Text-fig. 8). About this stage, the two valves are pushed apart by the enlarging protoplast which then becomes enveloped in a thin membrane, the perizonium (Text-figs. 8 and 12). Two of the four nuclei degenerate, while the other two fuse (Pl. X, Fig. 6; Text-fig. 9). After the fusion of the two nuclei (Pl. X, Fig. 7; Text-fig. 10), the protoplast increases very much in size (Pl. X, Fig. 8; Text-fig. 11). The protoplast then contracts away from the perizonium on one side first and secretes a valve, the epitheca, on this side (Pl. X, Fig. 9; Text-fig. 13). Later it contracts away from the other side also and soon the second valve, the hypotheca, is formed. The new cell (Text-fig. 14) which is nearly three times as large as the old mother-cell becomes finally free after the rupture of the perizonium.
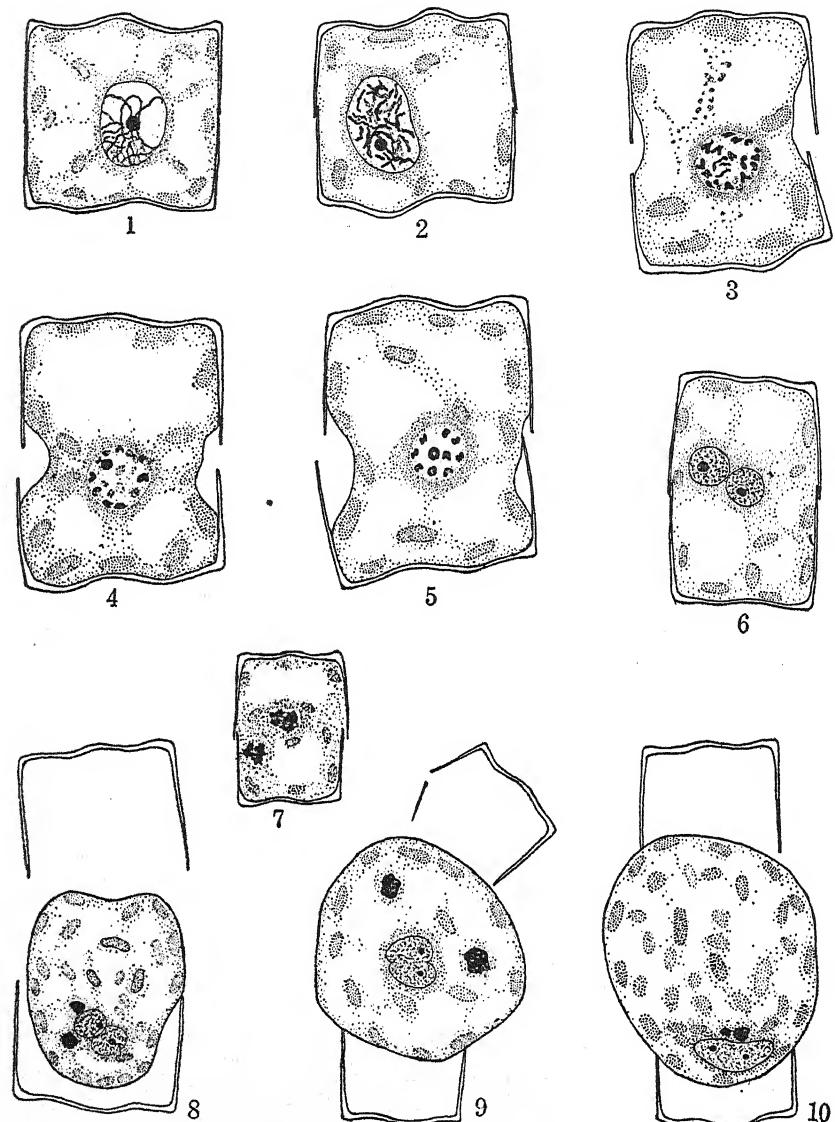
Text-figs. 1–10. *Cyclotella Meneghiniana* Kütz.—Fig. 1. Synizesis. Fig. 2. Pachytene. Figs. 3–5. Diakinesis in three different foci. Fig. 3. Upper focus. Fig. 4. Median focus showing nucleolus. Fig. 5. Lower focus. Fig. 6. Two-nucleate stage after first division of meiosis. Fig. 7. Anaphase of second division. Fig. 8. Four-nucleate condition of the auxospore with two normal nuclei and two degenerating nuclei. Note the valves just pushed apart by the enlarging auxospore. Fig. 9. Auxospore with two normal nuclei just fusing and the remaining two degenerating. Fig. 10. Auxospore showing the fusion nucleus and the two degenerated nuclei. Note the two nucleoli in the fusion nucleus. All figures (× 1400).

The results of the present investigation may be summed up briefly as follows. The vegetative phase in *Cyclotella Meneghiniana*
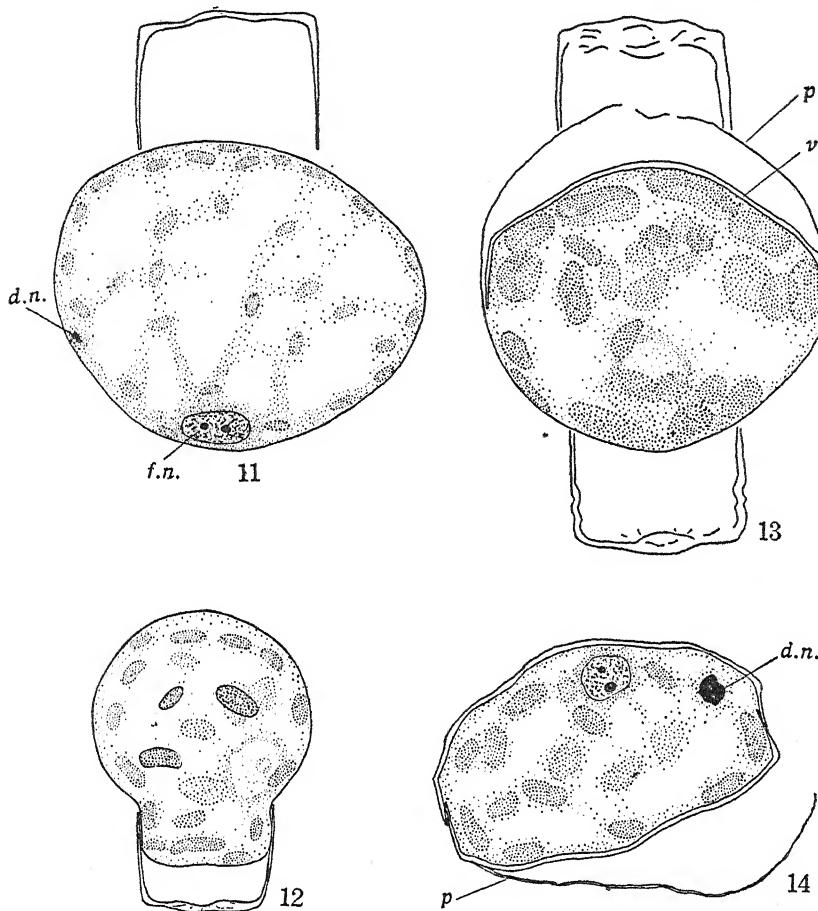
Text-figs. 11–14. *Cyclotella Meneghiniana* Kütz.—Fig. 11. Auxospore very much increased in size showing the fusion nucleus and one of the two degenerated nuclei still persisting. Note one of the valves has already been pushed off (× 1400). Fig. 12. Enlarging auxospore drawn from a living specimen. Note one end of the auxospore is still inside a valve, the other valve having been thrown off already (× 1060). Fig. 13. Formation of the first valve inside the perizonium (× 1400). Fig. 14. The new cell showing both the valves fully formed with a portion of the perizonium still attached at one side. Note one of the two degenerating nuclei still persisting (× 1400). (*d.n.*—degenerating nucleus; *f.n.*—fusion nucleus; *p.*—perizonium; *v.*—valve.)

is definitely diploid as in the Pennales. During auxospore-formation the nucleus undergoes two divisions, of which the first is reductional, and forms four nuclei. Of these four nuclei two fuse

and form the nucleus of the auxospore, while the remaining two degenerate. Auxospore-formation here is clearly the result of a definite sexual process brought about through the autogamous fusion of two surviving gametic nuclei and conforms to Type III *b*, of Geitler among the Pennales (Geitler, 1932, p. 213 ; 1935, p. 155). These observations of the authors on *Cyclotella Meneghiniana* are in full agreement with those of Persidsky made on *Melosira varians* (Persidsky, 1935). But Persidsky was not able to observe all the stages of the heterotypic division in *M. varians*. He was able to observe only synizesis, late anaphase and telophase. The authors, however, were able to observe in *Cyclotella Meneghiniana* almost all the characteristic stages of the heterotypic division.

Until now, as Geitler (1935) has rightly pointed out, only in one member of the Centrales, viz., *Melosira varians*, are the details of auxospore-formation and nuclear alternation known fully. The present investigation adds one more member of the Centrales, viz., *Cyclotella Meneghiniana* Kütz., in which these details are completely known.

The facts brought out by the present and other recent investigations already mentioned, would appear to suggest that there is not much fundamental difference between the Pennales and the Centrales. It is very desirable that more members of the Centrales are investigated in detail as regards auxospore-formation.

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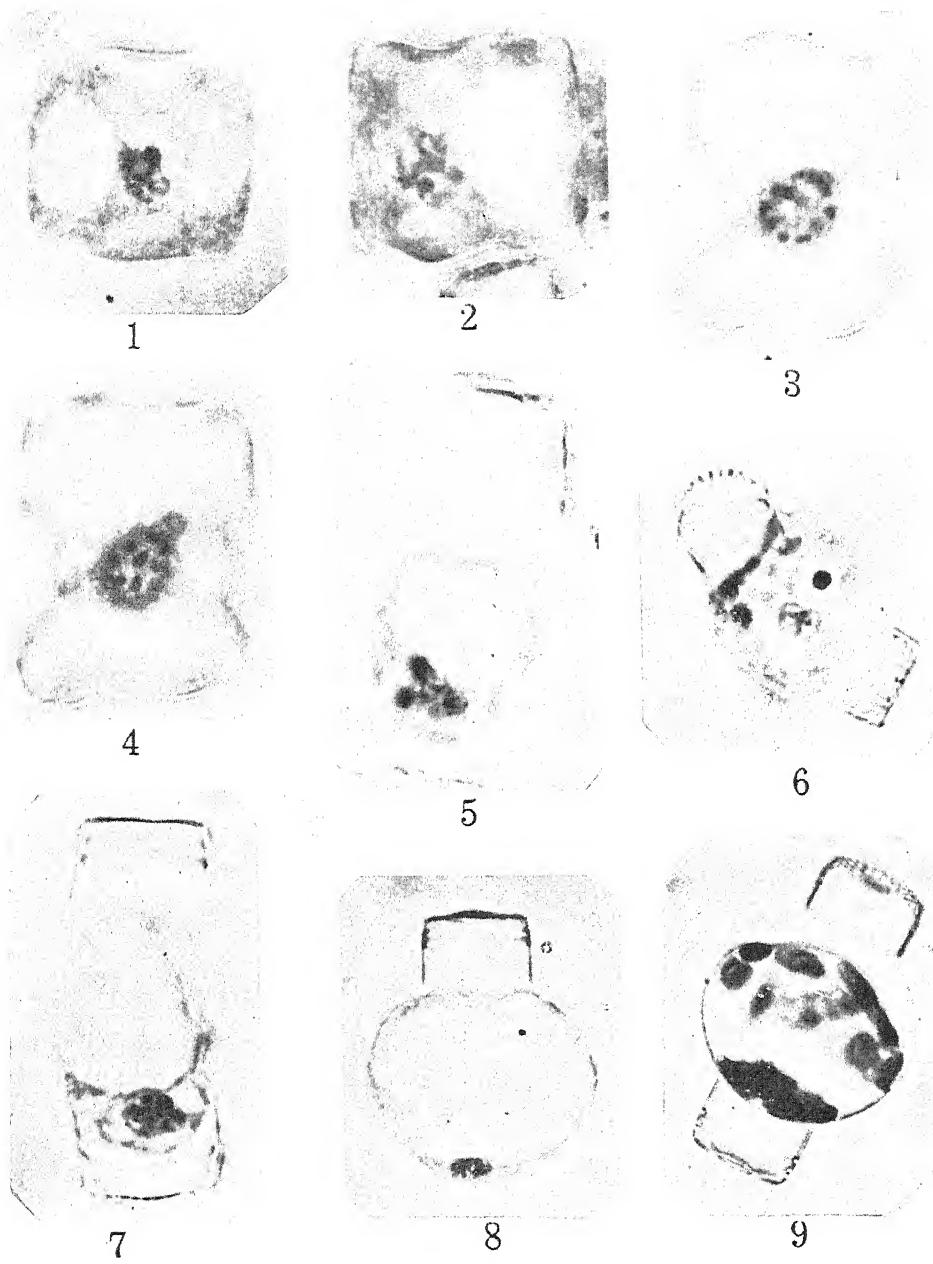
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EXPLANATION OF PLATE X

Cyclotella Meneghiniana Kütz.

FIG. 1.   Synizesis ($\times$ 1650).

FIG. 2.   Pachytene ($\times$ 1650).

FIG. 3.   Diakinesis: median focus showing the bivalents and the nucleolus ($\times$ 1650).

FIG. 4.   Same as the above taken at a still lower focus ($\times$ 1650).

FIG. 5.   Auxospore showing the four-nucleate condition with two normal and two degenerating nuclei. (The darkly stained masses represent the degenerating nuclei) ($\times$ 1460).

FIG. 6.   Auxospore showing the fusion of the two nuclei. Note the two degenerating nuclei are seen as darkly stained masses ($\times$ 1050).

FIG. 7.   Auxospore showing the fusion nucleus and the two degenerating nuclei ($\times$ 1310).

FIG. 8.   Auxospore increased very much in size, showing the fusion nucleus ($\times$ 825).

FIG. 9.   Photomicrograph of a living auxospore showing the formation of one of the two valves inside the perizonium ($\times$ 825).

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THE DEVELOPMENT OF THE EMBRYO SAC IN *HECKERIA UMBELLATA*, KUNTH

By P. Maheshwari and H. Gangulee

Dacca University

Introduction

The only previous work on *Heckeria umbellata* is by Johnson (1902) who reported an *Adoxa*-type of embryo-sac in this plant. From a study of his figures, one of us (Maheshwari, 1937) concluded that the development is more likely of the *Fritillaria*-type. The present study was undertaken with a view to verify this supposition and test its veracity.

Material and Methods

The material was collected by the late Dr. Winfield Dudgeon from Chicago and fixed in formalin-alcohol. The paraffin blocks, prepared in 1916 were available to us through the courtesy of Mrs. Dudgeon and Dr. C. H. Rice, Principal, Ewing Christian College, Allahabad. The spadices were cut transversely at 7-10μ on a Spencer rotary microtome and stained in iron-hæmatoxylin.

Investigation

Ovary.—A description of the ovary has already been given by Johnson and we have nothing to add to his observations in this respect.

Ovule.—The nucellus arises from the base of the ovary and the two integuments close over it in the normal fashion (see figs. 1, 2, 8 and 15). The outer integument is mostly 2-layered but the cells are narrow and shrunken.

The inner integument is 3-layered in the beginning but becomes thicker in older stages.

Embryo-Sac.—The hypodermal archesporial cell cuts off a primary wall cell towards the outside which undergoes periclinal divisions (fig. 1) to produce a parietal tissue which is about 4 cells
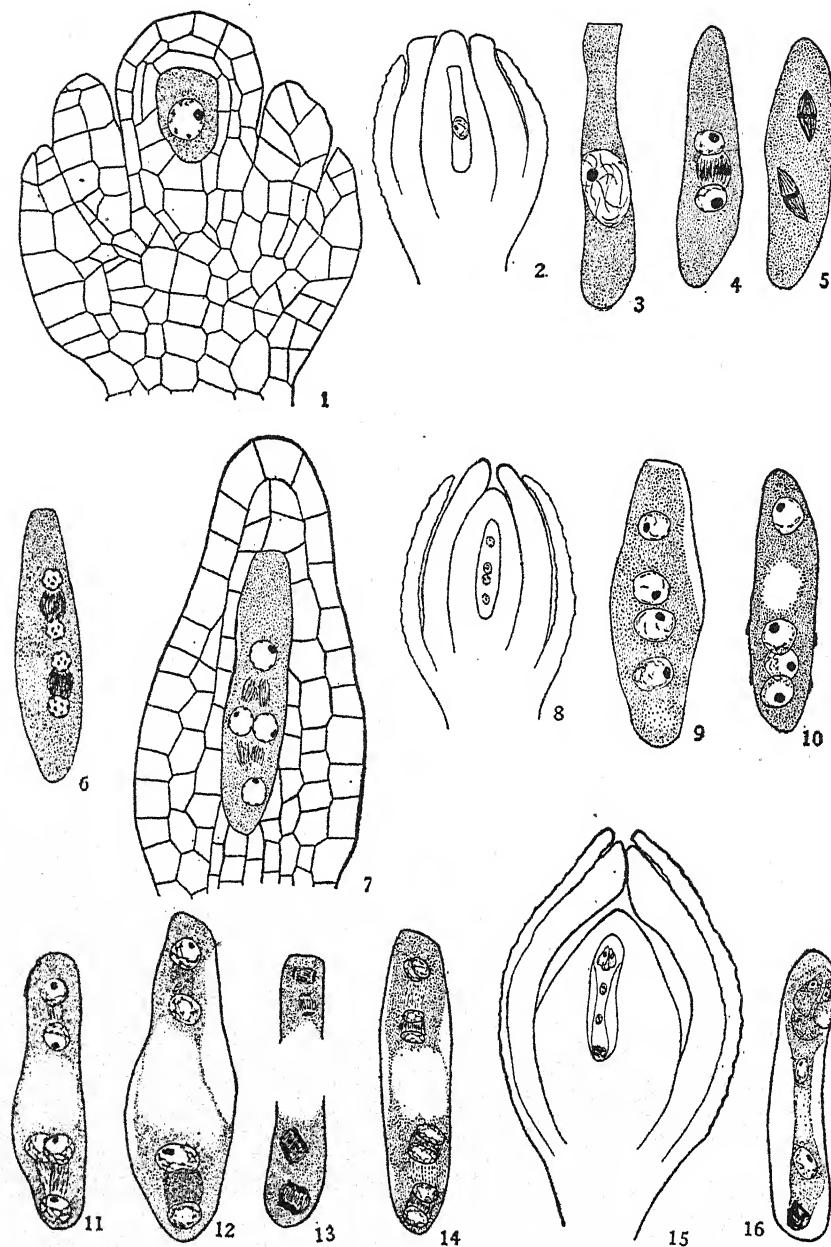
Fig. 1. L. S. ovule showing a young megaspore mother cell. × 760.
Fig. 2. L. S. ovule (diagrammatic) at the megaspore mother cell stage.
× 260. Fig. 3. Megaspore mother cell, preparatory to meiosis. × 760.
Fig. 4. Two-nucleate stage, resulting from first division of the mother

deep.   The nucleus of the megaspore mother cell (fig. 3) goes through
the usual premeiotic changes and divides to form 2 daughter nuclei
without an accompanying wall formation (fig. 4).   Both the nuclei
divide again (figs. 5,6) to form the 4 megaspore nuclei which
become interconnected by spindle fibres but do not show any
intervening walls (fig. 7).   At first the 4 nuclei are arranged cross-
wise and are almost equally distant from each other.   The micro-
pylar nucleus continues to remain in its original position or even
moves up slightly; the 3 remaining nuclei on the other hand show
a distinct tendency to migrate downward (fig. 9).   Soon a vacuole
appears between the two groups resulting in a 1 + 3 arrangement,
such as is characteristic of the *Fritillaria*-type (Bambacioni, 1928).
This stage was encountered so many times in our preparations
that we are certain about its being a normal phase in the life-cycle.

The next division was not observed but a stage like that in
fig. 12 leaves no doubt in our mind that while the micropylar
nucleus divides alone in the normal way, the 3 chalazal nuclei or
their spindles unite before the division is completed.   Thus the
number of nuclei after the division remains as before, except that
the two micropylar nuclei are small and haploid, while the 2 chala-
zal ones are distinctly larger and triploid.   While we did not get
any stage in our material to permit an actual chromosome count,
the difference in size between the micropylar and chalazal nuclei
was noticed in every section showing this particular stage.
The next division (fig. 13) in which actual spindles were seen, shows
a clear difference in the number of chromosomes in the upper and
lower halves of the embryo-sac.   The counts that we made in a
preparation showing metaphase chromosome plates (not figured)
definitely proved that the lower spindles had *more than twice* the
number present in the upper.[1]

Fig. 14 shows the telophase of the last division in which again
the larger size of the chalazal nuclei is distinctly seen.   The mature
embryo-sac (figs. 15, 16) is of the usual organisation but most of
those seen in our sections were already on their way to degeneration.

Fig. 11 is an exceptional case in which the micropylar mega-
spore nucleus has divided but the 3 chalazal nuclei have remained
undivided.

cell. × 760. Figs. 5 & 6.   The two nuclei dividing. × 760. Fig. 7.
L. S. nucellus showing the four megaspore nuclei just after reduction
divisions are over.   × 760.   Fig. 8.   L. S. ovule (diagrammatic) at
4—nucleate stage.   × 260.   Fig. 9.   Embryo-sac showing the 4 megaspore
nuclei arranged in a single row.   × 760. Fig. 10.   Megaspore nuclei
showing a 1 + 3 arrangement.   Note the intervening vacuole.   × 760.
Fig. 11.   An abnormal condition in which the micropylar nucleus has
divided but the 3 chalazal ones are as before. × 760. Fig. 12. Secondary
4-nucleate stage. × 760. Figs. 13 & 14.   Last division in the embryo-
sac.   × 760.   Fig. 15.   L. S. ovule (diagrammatic) at the 8-nucleate
stage.   × 260.   Fig. 16.   Mature embryo-sac. × 760.

[1] Due to the clumping of chromosomes caused by unsatisfactory
fixation the exact number could not be ascertained, however.

DISCUSSION

There is now no doubt that the development of the embryo-sac in *Heckeria* is of the *Fritillaria-* and not the *Adoxa*-type. Johnson's drawings are quite accurate but he does not seem to have given adequate attention to the proper sequence of stages and dismisses the matter in a couple of lines :—" This single megaspore,[2] after enlarging slightly, gives rise to two, four, and finally eight nuclei in the typical manner ". His fig. 21 can now be interpreted as showing the megaspore nuclei just passing into the $1+3$ arrangement while fig. 20, considered by him to be younger, is actually the older stage and shows the telophase leading to the secondary 4-nucleate stage. Häuser's (1916) fig. 31 of *Piper subpeltatum* is essentially similar to our fig. 13 and must also be interpreted on the same lines.

SUMMARY

The development of the embryo-sac of *Heckeria umbellata* corresponds to the *Fritillaria*-type. Johnson's interpretation of an *Adoxa*-type in this plant is shown to be incorrect.

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[2] He really means the megaspore mother cell.

ANATOMICAL STUDIES IN THE LEAVES
OF THE MILLETS

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Introduction

The principal millets of South India comprise six genera and eight species, growing mostly under 'rain-fed' conditions. Though these have been studied from time to time by various authors along with many others in the general surveys of the Anatomy of the Grass-leaves, a detailed record handy enough for a breeder is wanting, and with that object is the present study undertaken.

Pee-Laby (1898) has reviewed in detail the older literatures up to his time. It is interesting to note that the opinion of Linnæus influenced the botanical work so much in these times, that no further work was undertaken till some German botanists broke fresh ground in 1865. The natural inclination was to try to classify plants based on anatomy.

Duval-Jouve (1875) was the first **to** undertake a comparative study of the **ana**tomy of the leaves of the Grasses.

He deals the histology of the leaves under the following heads :

III. Histology.

    1. Epidermis.

        (*a*) Bands masking the hypodermal fibrous tissue,

        (*b*) Bands masking the parenchyma,

        (*c*) Strips of bulliform cells.

    2. Fibrovascular bundles.

    3. Hypodermal fibrous tissue.

    4. Parenchyma.

        (*a*) Parenchyma of Chlorophyll,

        (*b*) Common colourless parenchyma,

        (*c*) Stellar parenchyma.

Under each head he gives a further classification of the characters. The species examined by him are classified under each of the sub-groups.

After him came Schwendener who studied the stomata (1889) and 'Mestomscheiden' (1890) in the leaves of the Grasses.

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Beal (1896) gave a general anatomical consideration of the North American grasses. He classified the occurrence of the Motorcells which he calls "Bulliform cells or Blister cells". The only cultivated species treated is *Zea mays*.

Following Duval-Jouve, Pee-Laby (1898) worked out in great detail the grasses of France, laying more stress on the habitat, etc. He treats of the leaf under (1) Epidermis, (2) Mesophyll, (3) Nerves, (4) Green-sheath, (5) Mechanical tissue, and (6) Motor-tissue. He divides the grasses in 4 groups according to (1) the parallel or un-parallel nature of the two surfaces, (2) the number of stomata on each surface, (3) the green parenchyma, and (4) Motor-cells. The green sheath and the green parenchyma in general have been treated in detail.

Marshall Ward (1901) has studied the anatomy in relation to the habitat. He has tried to classify the grasses according to (1) chlorophyllous tissue, (2) presence or absence of lacuneæ around vascular bundles, (3) the nature of the upper and lower epidermis, (4) motor cells, and (5) hairs.

Lewton-Brain (1901-1905) while recognising the aid given by the anatomical studies to Systematics, stresses the fact that the environment plays a greater part in determining the structure, and that too much reliance should not be placed on this evidence. He further notes four main types in the grass leaves according to the character of the surface. He records the difference in the appearance of the arrangement of the cells in longitudinal sections from the transverse.

Warming (1909) and Armstrong (1917) have correlated the anatomy of the leaves with the habitat. Stapledon (1912–14) comes to the conclusion that the phenomena of drought resistance is not associated with any one set of morphological characters but on the ' Growth-form ' and ' Plant habit '. Sabnis (1921) deals chiefly with the fibrovascular tissues, the leaf surfaces and the distribution of the stomata. Rangachari (1921) gives a detailed description of some typical wild grasses.

Avdulow (1931) coupling leaf-anatomy with cytological data finds that the grasses could be grouped into two main types :– 'Typus I mit der karnzformigen Disposition des assimilatorischen Gewebes ' and the ' Typus II : das Chlorophyllführende Parenchym den ganzen Raum zwischen den Bündel ausfüllt und nur in einigen Fällen farblosem Parenchym oder Lufträumen Platz macht'. The former is characteristic of grasses from tropics or subtropics, while the second of the temperate and sub-temperate, and also of some of the more primitive grasses as *Bambuseæ*, *Oryzeæ*, etc. The type I is considered as derived from Typus II. Further they are correlated with the chromosome number and the starch grain-form. The groups *Saccharifereæ* with simple starch grains, Type I in leaf anatomy and small chromosome with 9 or 10 as the basic number and the *Poateæ* with compound starch grains, Type II in leaf

anatomy and large chromosomes with 7 as the basic number are derived. It is further noted that the type I goes with the vertical position of the first seedling leaf and type II with a more horizontal position.

A very detailed work on the epidermis and epidermal structures of grasses in general and more in particular of the families *Aveneæ, Hordeæ* and *Agropyrum*, etc., was published by Pratt (1932). He has worked mostly with surface views of whole mounts obtained by a special technique.

Arber (1934) has used the leaf anatomy as one of the data in discussing the evolution of the gramineæ.

Amongst cultivated cereals Kiesselbach (1916) and Weatherwax (1923) have studied the anatomy of *Zea mays*. Kiesselbach (1916), Miller (1916) and Miller and Coffman (1918) have compared the leaves of Sorghum with corn in connection with drought resistance. Percival (1921) has examined the leaves of the wheat plant and Artschwager (1921) of sugarcane. Tullis (1935) has studied the anatomy of the rice leaves in connection with the attack of *Helminthosporium oryzeæ*.

MATERIALS AND METHODS

Mature leaf blades alone have been treated. The specimen in each leaf was taken about the middle and fixed in Carnoy's fluid. Paraffin sections both transverse and longitudinal (sections cut in such a way as to pass through both the epidermis and the vascular bundle and at the same time parallel to the margins and not in such a way as to pass through both margins and the knife parallel to the epidermis) were cut at 10μ and stained in Haidenhain's Hæmatoxylin as also in Picro-anilin-blue. Fresh sections of the leaves were also examined for comparison. All drawings and measurements were done from the fixed and stained materials. The thickness of the leaves were measured at the place where the midrib ended and the blade really began. All photomicrographs were done with a Zeiss microscope and Beck's photomicrograph quarter plate camera. Unless otherwise mentioned all the figures are magnified ×350.

The following species were examined:—

1. *Andropogon Sorghum*, Brot. (*Sorghum durra*)
2. *Pennisetum typhoides*, Stapf and Hubbard
3. *Setaria italica*, Beauv.
4. *Paspalum scrobiculatum*, L.
5. *Echinochloa colona*, Link. var. *frumentacea* C. E. C. Fischer.
6. *Panicum miliare*, Lam.
7. *Panicum miliaceum*, L.
8. *Eleusine coracana*, Gaertn.

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OBSERVATIONS

The millets show a great uniformity with regard to the shape of their leaf blades. They are lanceolate to linear-lanceolate. The tips of the leaves are either bluntly or acutely pointed. The blade in all of them is thickest on either side of the midrib and gets thinner towards the margins.

The epidermis in all the millets consists of a single layer of cells oval or rectangular in transverse sections and in longitudinal sections mostly rectangular, with the longer axis arranged parallel to the length of the leaf. The walls are thickened at the outer side. They are reduced in size over a fibrovascular bundle. Alternating with the long cells are found small isodiametrical ones. The hair cells arise from single small cells. The hairs are trichome-like, curved or filiform.

The motor cells are found on the upper surface. They are always larger than the ordinary cells, and thinner walled. The cells are narrower on the outer than on their inner side (Fig. 2).

The stomata are constructed on a very general plan typical in grasses. They occur on both surfaces, alternating with the bundles and in the upper row in the neighbourhood of the motor cells. They are more numerous on the lower surface than on the upper.

The mesophyll consists of fibrovascular and chlorophyllous tissues. The vascular tissue is of the collateral, closed type, inverted and with pronounced phloem (Fig. 1). In all the millets the large and the small bundles are found inter-mixed. The smaller bundles differ from the large ones in the absence of the protoxylem and the two large mataxylems. All the millets possess a mestome sheath in the large bundles.

A bundle sheath with chloroplasts is present in all the millets. These cells are larger in the smaller bundles but more numerous in the larger ones. The cells in transverse sections appear narrower on the inner side than on the outer, and bulge out on the outer side. The smaller bundles alone are completely enclosed by the sheath, while the larger ones have sclerenchyma beneath both epidermis.

The mechanical tissue is found as I-girders and as a band at the margin of the leaf (Fig. 4). The fibrous cell is so highly sclerified that the lumen is almost closed. The quantity of mechanical tissue is reduced in the smaller bundles.

There is little or no variation in the arrangement of the chlorophyllous tissues. In transverse sections it is composed of girdles round the vascular bundles and of the loose tissue connecting the two girdles. The cells of the girdle tissue are elongated and compact while those of the middle are large, loose, irregularly shaped and often branched. The chlorophyllous tissue extends to the two

epidermis but their continuity is broken by the occurrence of the sclerenchyma tissue.

In longitudinal sections the leaves show a regular row of cells running under either epidermis or sometimes under both (Fig. 3). In most cases the upper epidermis consists of one or two layers of palisade-like cells sometimes compact but commonly with intercellular spaces and often branched. Below this there are two to three layers of loose, branched irregularly shaped cells also with much intercellular space. On this may be found one or sometimes two layers of elongated cells somewhat compact. The latter tissue is immediately adjacent to the lower epidermis. Whenever a vascular bundle without sclerenchyma occurs, the chlorophyllous tissue occupies the sides of the bundle. In such cases it is composed of one or two layers of elongated rather compact cells. In cases where heavy sclerenchyma occurs the chlorophyllous tissue is absent.

The midrib is roughly semilunar in shape. It consists of a number of bundles both large and small, the number depending on the size of the midrib. The chlorophyllous tissue also occurs, disposed as in the blade. The sclerenchyma tissue of each bundle is increased in quantity even in the non-sclerified ones. Both the above tissues are confined to the lower surface. The upper sclerenchymatous tissue occurs united into a band along the upper epidermis. The motor tissue is absent in all the midribs.

Apart from the above common features, the blades show certain characters which differentiate them from one another, of which the following are the more important:—

1. *Size of leaf* (arranged in order of length of leaf; the area is calculated taking the leaf as a triangle).—

Species	Length in cm.	Width in cm.	Thickness in mm.	Area
1. *P. typhoides*	<u>70</u>	5·0	0·31	105
2. *A. sorghum*	60	<u>7·5</u>	0·20	225
3. *E. coracana*	54	1·5	<u>0·37</u>	40·51
4. *P. miliare*	50	1·5	0·24	37·5
5. *E. colona* var. *frumentacea*	45	3·0	(0·17)	67·5
6. *S. italica*	45	3·5	0·20	78·75
7. *P. scrobiculatum* ..	45	(1·2)	0·30	27·0
8. *P. miliaceum*	(33)	1·5	0·22	24·75

(The highest values are underlined and the lowest are bracketed)

2. *Shape, e'c.—*

	Species	Leaf margin	Position of blade in relation to midrib	Texture
1.	*A. sorghum* ..	Usually wavy	Slightly grooved	Smooth
2.	*P. typhoides* ..	do.	do.	do.
3.	*E. coracana* ..	Straight	Folded	Slightly rough
4.	*P. scrobiculatum* ..	do.	Slightly grooved	do.
5.	*P. miliare* ..	do.	do.	Rough
6.	*E. colona* var. *frumentacea*	do.	do.	do.
7.	*P. miliaceum* ..	do.	Fairly flat	Slightly rough
8.	*S. italica*	do.	do.	Rough

3. *Epidermis.—*

(*a*) Outer wall smooth except for hairs: *A. sorghum; P. typhoides; E. coracana; E. colona* var. *frumentacea ; P. miliare* and *P. miliaceum.*

(*b*) With blunt or gland-like projections: *S. italica.*

(*c*) Outer walls project slightly and are free : *P. scrobiculatum.*

4. *Surface in transverse sections.—*

(*a*) The two surfaces show very gentle undulations at great intervals : *A. sorghum; P. typhoides; E. colona* var. *frumentacea; P. scrobiculatum.*

(*b*) Upper surface more undulating than lower : *E. coracana; P. miliaceum.*

(*c*) Surfaces show distinct ridges and furrows : *S. Italica; P. miliare.*

5. *Motor tissue* (Fig. 2).—

(1) Number.—

(*a*) Not definite : *A. Sorghum ; P. typhoides ; S. Italica ; P. scrobiculatum ; E. colona* var. *frumentaceum.*

(*b*) Rather definite (about 3) ; *P miliare ; P. miliaceum ; E. coracana.*

(2) Shape.—

(*a*) Not very distinct: *A. sorghum; S. italica; P. scrobiculatum; E. colona* var. *frumentaceum.*

(*b*) More distinct than in (*a*), cells larger and outer side narrower : *P. typhoides.*

(*c*) Very distinct ; a central large pyriform cell and two smaller similarly shaped cells on either side of the central : *P. miliare P. miliaceum*; *E. coracana.*

6. *Stomata.—*

Species	Size in μ		Position in relation to other epidermal cells	
	Length	Width	Upper	Lower
P. typhoides	38	32	Slightly raised	Slightly raised
A. sorghum	34·8	22·4	At level	At level
E. coracana	30	20	do.	Below level
P. miliaceum	29	20	Slightly below	Level
P. scrobiculatum ..	28	20	Below	Below
E. colona var. frument-acea	26	20	Slightly below	Slightly below
P. miliare	25	13	Level	Level
S. italica	20	16·8	Slightly below	do.

7. *Fibrovascular tissue* (**Fig. 1**).—

(*a*) Three sizes of bundles (Primary—largest Secondary—smaller than primary; and Tertiary—smallest. Only the first two are stereomed) : *A. sorghum* ; *P. typhoides* ; *S. italica*; *P. scrobiculatum*; *E. colona* var. *frumentacea.*

(*b*) Only two kinds of bundles—the primary and the secondary both with stereome : *E. coracana* ; *P. miliaceum* ; *P. miliare.*

8. *Chlorophyllous tissue.—*Varies only with regard to the length of the girdle cells. Bundle sheath cells fall into :

(*a*) With small plastids, arranged along the outer wall : *A. sorghum*; *P. typhoides*; *S. italica*; *P. scrobiculatum*; *E. colona* var. *frumentacea*; *P. miliare* (**Figs. 1, 2, & 3**).

(*b*) With large plastids arranged along the inner wall : *E. coracana*; *P. miliaceum.* (**Figs. 2 and 5**).

9. *Size of plastids* (given in μ).—

Species	Parenchyma		Bundle-sheath	
	Length	Width	Length	Width
P. scrobiculatum	6·0	4·5	7·5	5·5
P. miliare	5·2	4·4	5·8	3·8
S. italica	3·6	2·8	7·5	4·2
E. colona var. *frumentacea* ..	3·6	3·0	4·0	3·7
A. sorghum	3·6	2·8	4·3	4·0
P. typhoides ·	3·2	2·2	5·8	4·2
P. miliaceum	5·6	3·6	22·4	7·4
E. coracana	3·8	2·2	25·6	7·6

10.   *Midrib.*—

(*a*) Only one primary vascular bundle: *E. colona* var. *frumentacea*; *S. italica*.

(*b*) More than one primary vascular bundles:

(1) With no lacuna: *A. sorghum*; *P. typhoides*; *P. miliare*; *P. miliaceum*; *E. coracana*.

(2) With one central lacuna: *P. scrobiculatum* (Fig. 6).

DISCUSSION

The millets as is well known are tropical and sub-tropical in distribution and secondly are all dry-farm crop plants. When we consider the natural distribution of these millets over South India it is seen that their greatest cultivation is confined to the plateau regions. In the plains they are confined to the drier areas and in the rich alluvial tracts they yield place to rice and other crops. The best display of *sorghum, pennisetum* and *setaria* lies in the dry black-cotton soil areas. The millets thus are from the very nature of their habitat subjected to great variations of rain, moisture and transpiration. Thus they find themselves in xeric environments of uncertain water availability and high transpiration. Some of the above marshalled data will be discussed from this point of view.

Weaver and Clements (1929) give three degrees of xeric leaves in the grasses: in the first the surfaces are almost smooth and parallel; motor cells not much developed, sclerenchyma very little, only on large and medium bundles. The second type has smooth furrows with large, not much defined motor cells, sclerenchyma on large and medium bundles, lower surface slightly grooved. In

the third and most xeric the motor cells are trinate, extending to lower surface, epidermis armed, all vascular bundles with sclerenchyma, both surfaces grooved. Sabnis (*l.c.*) finds the same characters in the desert grasses. The millets could be classified into these three groups as was seen in sections 4, 5 and 7. But it is also seen that *S. italica* most xeric as to surface, shows the least xeric motor tissue and on the other hand *E. coracana* and *P. miliaceum* medium xeric as to surface, show highly xeric motor tissue and sclerenchyma. *P. miliare* shows the surface similar to *S. italica*, motor tissue similar to *E. coracana* type but the sclerenchyma is as in *A. sorghum* type.

Further, Weaver and Clements (*l. c.*) describe **Zea mays** as having mesophytic leaves. They point out that the mesophytic leaves are characterised by the maximum development as to size, moderately thick, epidermis thin and transparent, chlorophyll abundantly developed and colour of leaf deep green. The millets are not strictly comparable among themselves as to size of leaf. They differ much as to habit, while *A. sorghum* and *P. typhoides* are generally tall, erect-growing plants not given to much of tillering, the rest of them are short and bushy in nature. The former two have large leaves and conform to the mesophytic type of leaf. But the leaf of *A. sorghum* which is nearly $1\frac{1}{2}$ times bigger than that of *P. typhoides*, is thinner and has smaller bundle sheath plastids. Taking the smaller leaved millets *S. italica* shows the largest leaf area but otherwise is least mesophytic while *E. coracana* shows a smaller leaf-size but has the thickest leaves and has large bundle-sheath chloroplasts, and *P. miliaceum* which is similar to *E. coracana* in chloroplasts, etc., has the smallest leaf area.

Though the epidermis in all the millets is armed with hairs, silicious cells, etc., *S. italica*, *P. miliare*, and *E. colona* var. *frumentacea* are outstandingly rough.

Taking the size of the stomata into consideration, the largest leaved species are seen to have larger stomata than the smaller leaved ones. The larger stomata is characteristic more of mesophytic plants than of the xeric ones. Whether the two are correlated, could not be said as the number of stomata per unit area was not studied. However, amongst the small leaved types *S. italica* which had the largest leaf size shows the smallest stomata and *P. miliare* with the smallest leaf size shows the largest stomata, as also *E. coracana*.

The chloroplasts, however, are not in any way influenced by the size of the leaf, *P. scrobiculatum* and *P. miliare* have the largest plastids in parenchymatous cells. The width of the plastids, however, is proportional to the length, so that the plastids are more or less oval in the parenchyma cells of all the millets. The bundle sheath plastids seem to show no relation to the parenchyma plastids. *E. coracana* and *P. miliaceum* have the largest plastids. *S. italica* which has shown other extreme xeric characters, shows larger plastids than *A. sorghum*.

Thus it is seen that *A. sorghum* and *P. typhoides* show the least number of xeric structures, whereas *S. italica* and *E. colona*, var. *frumentacea* show the greatest number. No one species, however, shows all the xeric characters at the same time, but on the other hand xeric and non-xeric characters are found in all. It is thus possible, as pointed out by Weaver and Clements (*l.c.*) that these characters are to a great extent ancestral. These authors remark (p. 347) "various xerophytes have, moreover, often found themselves in conditions that changed them into mesophytes. Many of them have, in consequence, retained characters of leaf, stem, or root that are to be regarded as ancestral rather than as the result of adaptation to the plant habitat."

A. sorghum, P. typhoides, and *S. italica* are cultivated under conditions in which the others would not thrive, yet the former especially, *A. sorghum and P. typhoides,* show the least xeric characters. Miller and Coffman (1918) however have come to the conclusion that in most cases a small leaf surface is the important factor in reducing the loss of water. Miller (1916) found that the better drought resistance of the sorghums was due to a smaller leaf surface combined with a larger and more efficient root system as compared to maize. Kiesselbach (1916) found that anatomically sorghum leaves did not differ much from that of maize and that they do not have lower water requirement in the production of dry matter than does corn under favourable conditions. But the smaller leaved millets like *E. coracana, P. milaceum*, etc., would hardly grow under the same conditions as sorghum. Just why sorghum is better adapted to dry soils could not be said from leaf anatomy. Kiesselbach (*l.c.*, p. 205) points out "it is well known that some plants are especially adapted to dry and others to humid conditions. For instance, the grain sorghums are well suited to dry-farming. Just what characters fit them for this purpose is not known." It might be, as pointed out by him, that these plants possess a high osmotic pressure and are able as a consequence to extract water from a comparatively dry soil. He further records that the sorghums have a capacity to go into a dormant condition during unfavourable growth conditions and renew the growth activity without having been greatly injured when rains come. Thus it may be concurred with Stapledon's (1912–13, p. 150) remarks "it is perhaps dangerous to assign too great an importance to the possession of apparently useful modifications. A fair correlation is, however, seen to exist between a plant's *manner of resistance* and its *growth-form.*" The development of the xeric structures such as motor tissue, woody tissue, etc., help in the diminution of loss of water and injury to delicate tissues during desiccation. Warming (*l.c.*, p. 127) points out "....some of the structural features of growth-forms of land plants are of such a nature that while no one can doubt their connection with a dry environment, their utility to the plant is not obvious. Among features of this problematic nature may be mentioned lignification." Further he points out that lignified parts serve as water storage organs also and " It must,

however, be remembered that lignified parts withstand extreme temperatures better than watery thin-walled parts can, and that trees endure greater fluctuations in humidity than herbs do." Weaver and Clements (*l.c.,* p. 347*ff.*) give four types of xeric plants. " 1. drought escaping, 2. drought evading, 3. drought enduring, 4. drought resisting. " The adaptation of the leaves to a smaller water supply fall into (1) position of the leaf, (2) rolling or folding of the leaf, (3) reduction of the leaf surface or loss of leaves, (4) changes of epidermal cells, (5) modifications of the stomata, and (6) changes in chlorenchyma. Thus in the breeding of drought-resistant strains Kiesselbach's remarks (*l.c.,* p. 205) "The production of plants adapted to meet the requirements for a low transpiration rate offers a field of great possibilities. Under this may be included the testing and also selection of established varieties already well adapted, and also selection, within a variety, of strains possessing certain characters, or the creation by breeding of new characters correlated with low relative water consumption," seems to be very pertinent.

In concluding, a few remarks may be made on the behaviour of the bundle-sheath plastids. As was seen above, *E. coracana* and *P. miliaceum* have large club-shaped plastids, while the rest have small, oval ones. The difference in the arrangements was also noticed. These large plastids are found in other species also, *e.g.,* *E. Indica* a close relation of *E. coracana, Cynodon dactylon,* etc. The peculiar arrangement of the large plastids all bunched together by their narrower ends, was noticed by Pee-Laby (*l.c.*) also. With reference to *Cynodon dactylon* he remarks, " These clubs appear resting by their narrower part on the inner wall of the cell, they are immersed in the colourless liquid in the midst of which are found crystals of oxalate of lime, etc." With regard to their structure he observes that each of the clubs is formed of proto-plasmic stroma composed of granules of variable size, impregnated with chlorophyll. This is in agreement with the present obser-vations on the two species. Kiesselbach (*l.c.*), however, regards the arrangement and also the elongated shape of the plastids as abnormal and unnatural brought about by the fixing fluid. He notes, however, the distinct difference in size and reaction to the fixative, between the plastids of the parenchyma and those of the bundle-sheath cells. Artschwager (*l.c.*) also attributes the cres-cent-shaped arrangement to the fixing fluid. With a view to decide whether this peculiarity was only an artifact, fresh leaves of both *sorghum* and *E. coracana* were examined at different times of the day. At each examination fresh portions of the leaf from the plant were taken. It was found that about the early portions of the day, in *sorghum,* the plastids were grouped along the outer margins of the cells and towards noon many of them had begun to wander into the middle of the cell. Towards evening they had begun again to group themselves. The same behaviour was found in *E. coracana* also but the arrangements were found on the inner wall. Thus it was seen that the above behaviour could not be ascribed to fixation.

Even then the behaviour proves that the *Eleusine* type has pores or some permeable membrane on its inner wall, while the others have it on the outer wall. The regularity of the arrangements, however, speaks against its being purely an artifact. It seems probable that this character is more a specific one than one of mere chance, and may if pursued throw some light on the evolutionary position of the species in the group. It would be interesting to know if any of the members of *Maydeæ* and *Andropogoneæ* also show the large plastids and if they did then the relation of the size, position, and a knowledge of the ecological factors would be of great importance in deciding whether the large plastids and the arrangements on the inner wall is a reaction to particular ecological factors. Secondly the grasses having evolved and specialised to withstand drought and desiccation the highest evolved species could be expected to show only smaller plastids and the less evolved the larger ones.

As already stated Avdulov (*l.c.*) takes leaf anatomy as one of the main evidences in classifying the grasses. He finds a high correlation between the type of leaf anatomy and karyology, *i.e.*, all those possessing Type I. anatomy agreed with the karyotype of the *Sacchariferæ*. He remarks, particularly to the Type I leaf anatomy, " Diese Abhängigkeit ist in dem Grade beständig, dass ich, sobald mir der anatomische Bau des Blattes bekannt war, mit Sicherheit seine zytologische struktur voraussagen konnte, nämlich kleine Chromosomen und die Grundzahl 9 oder 10, selten 12. Bis jetzt ist mir noch kein Ausnahme vorgekommen ". Further, this type of leaf is characterised by vascular bundles of several sizes—" Die verschiedenen grossen primären und kleinen Leitbündel alternieren im Querschnitt des Blattes in der Weise nach einem primären meist viele kleinere Bündel folgen ". Whereas in the Type II leaf anatomy the primary bundles alternate with one, two or atmost four small bundles. On these evidences Avdulov-forms the main group *Sacchariferæ* and further brings in *Chlorideæ* under this group. The anatomical structures as seen in the cross section of the leaves of these eight millets belonging to six genera confirm the above conclusions. While in five of them three sizes of bundles could be made out in three of them, *viz.*, *P. miliare*, *P. miliaceum*, and *E. coracana* only two kinds of bundles were present. In all of them, however, the primary bundles were followed by a number of smaller bundles, the number becoming less towards the midrib. The affinity of the *Chlorideæ* with the *Sacchariferæ* is further emphasised by the close similarity of the bundle sheath plastids of *P. miliaceum* and *E. coracana*. In fact the cross sections of these two leaves are difficult of distinction but for the thickness of the leaf in *E. coracana*. These evidences supplement those put forth for transfer of the *Chlorideæ* from *Poaceæ* to *Sacchariferæ* 'Avdulov *l.c.*, Krishnaswamy, 1939).

SUMMARY

The comparative anatomy of the leaves of the eight millets, *A. sorghum*; *P. typhoides*, *E. coracana*; *S. italica*; *P. scrobiculatum*;

P. miliare; P. miliaceum, and *Echinochloa colona* var. *frumentacea*
(in both longitudinal and transverse sections) have been studied.

These millets show adaptations in various grades to a xeric
environment. No one species shows complete xeric characters
but all of them show xeric and non-xeric characters together.
Many of the xeric characters are considered to be probably
ancestral. It is also seen that the leaf anatomy does not give
any definite clue to identify drought-resistant varieties though some
of the characters such as leaf-area, stomatal distribution, epidermal
structures, specialisation of motor cells, lignification, etc., may
help in the breeding of resistant varieties.

The bundle sheath plastids are found to be either small, oval
and arranged on the outer wall or large, club-shaped and arranged
on the inner wall. This character is not, so far as seen here, corre-
lated with the size of the leaf. It is considered to be probably a
specific one, or an adaptation to particular ecological conditions.
Its significance could not be definitely indicated seeing that smaller
plastids are better suited to dry environment, and the specialisa-
tion in the *Gramineæ* is towards withstanding drought and desic-
cation.

The relation, of leaf anatomy towards phylogeny in grasses
is briefly discussed.

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EXPLANATION OF ILLUSTRATIONS IN PLATES

PLATE XI

FIG. 1–2 *d*. FIG. 1. *Vascular bundles.*—(a) Primary (*P. typhoides*); (b) Secondary (i. *P. typhoides*, ii. *A. sorghum*); (c) Tertiary (*A. sorghum*—see also arrangement of bundle-sheath plastids); (d) Circular primary; and (e) Triangular smaller bundles of *P. miliare* (note bundle-sheath chloroplastids).

FIG. 2. *Motor tissues.*—(a) *A. sorghum* (×700) (note bundle-sheath plastids); (b) 1 and 2. *typhoides* (note bundle-sheath plastids); (c) *S. italica*; (d) *P. miliaceum* (note bundle-sheath plastids).

PLATE XII

FIGS. 2e.–6. FIG. 2. *Motor tissues.*—(e) *P. miliare*; (f) *E. coracana*.

FIG. 3. *Longitudinal section of leaf.*—(a) Through non-vascular portion (i. *A. sorghum*, ii. *E. coracana*); (b) Through vascular portion (i. *A. sorghum*, ii. *E. coracana*).

FIG. 4. *Mechanical tissue at the leaf margin.*—(a) *A. sorghum*; (b) *P. typhoides*; (c) *S. italica*.

FIG. 5. (a) Bundle-sheath plastids of *E. coracana*; (b) One bundle-sheath cell enlarged (×700).

FIG. 6. The midrib of *Paspalum scrobiculatum* (×90).
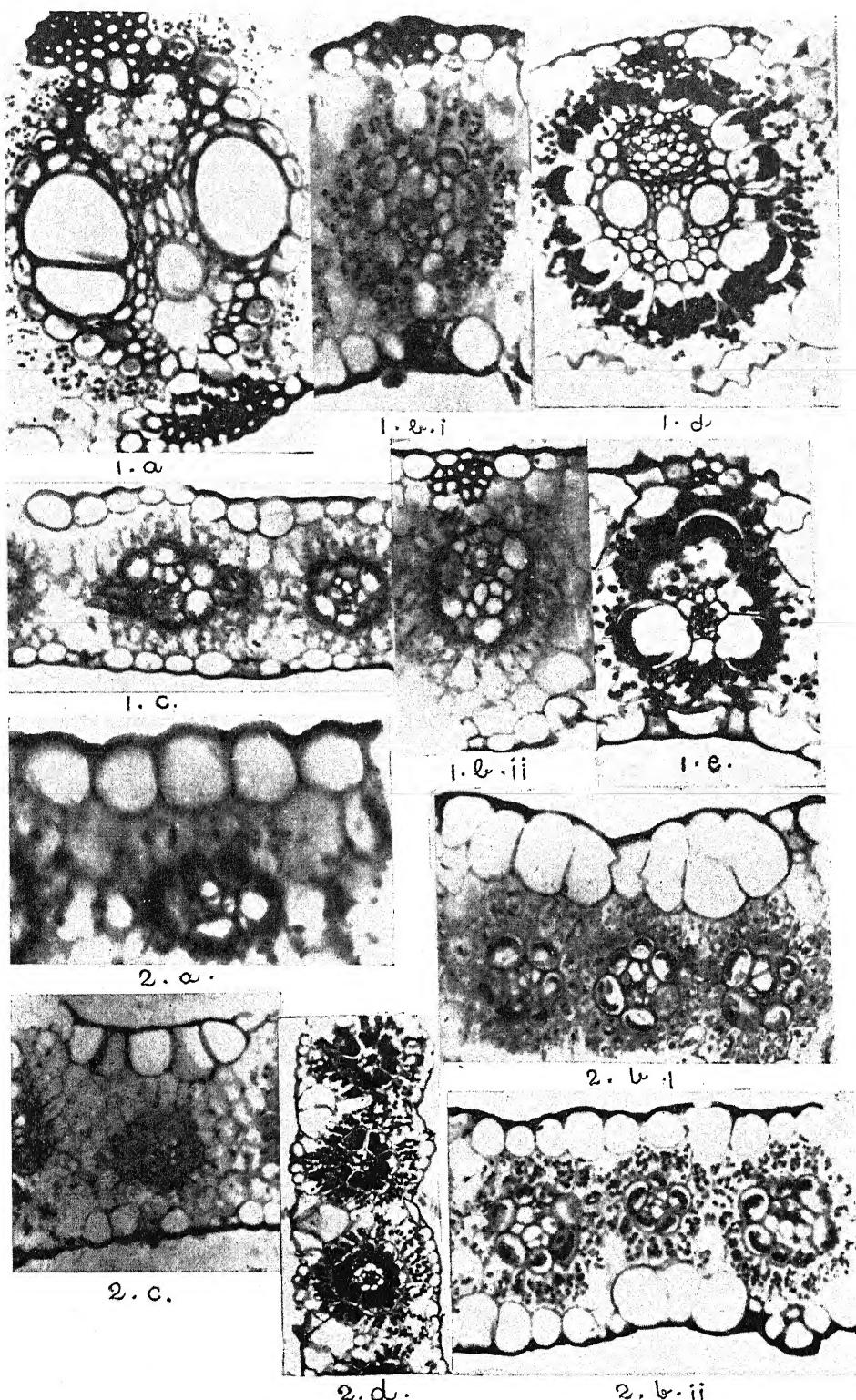
1. a.

1. b. i

1. d.

1. c.

1. b. ii

1. e.

2. a.

2. b. i

2. c.

2. d.

2. b. ii

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2.e.

2.f.

4.a.

4.c.

4.b.

3.a.i.

3.b.i.

3.a.ii.

3.b.ii.

5.a.

5.b.

6.
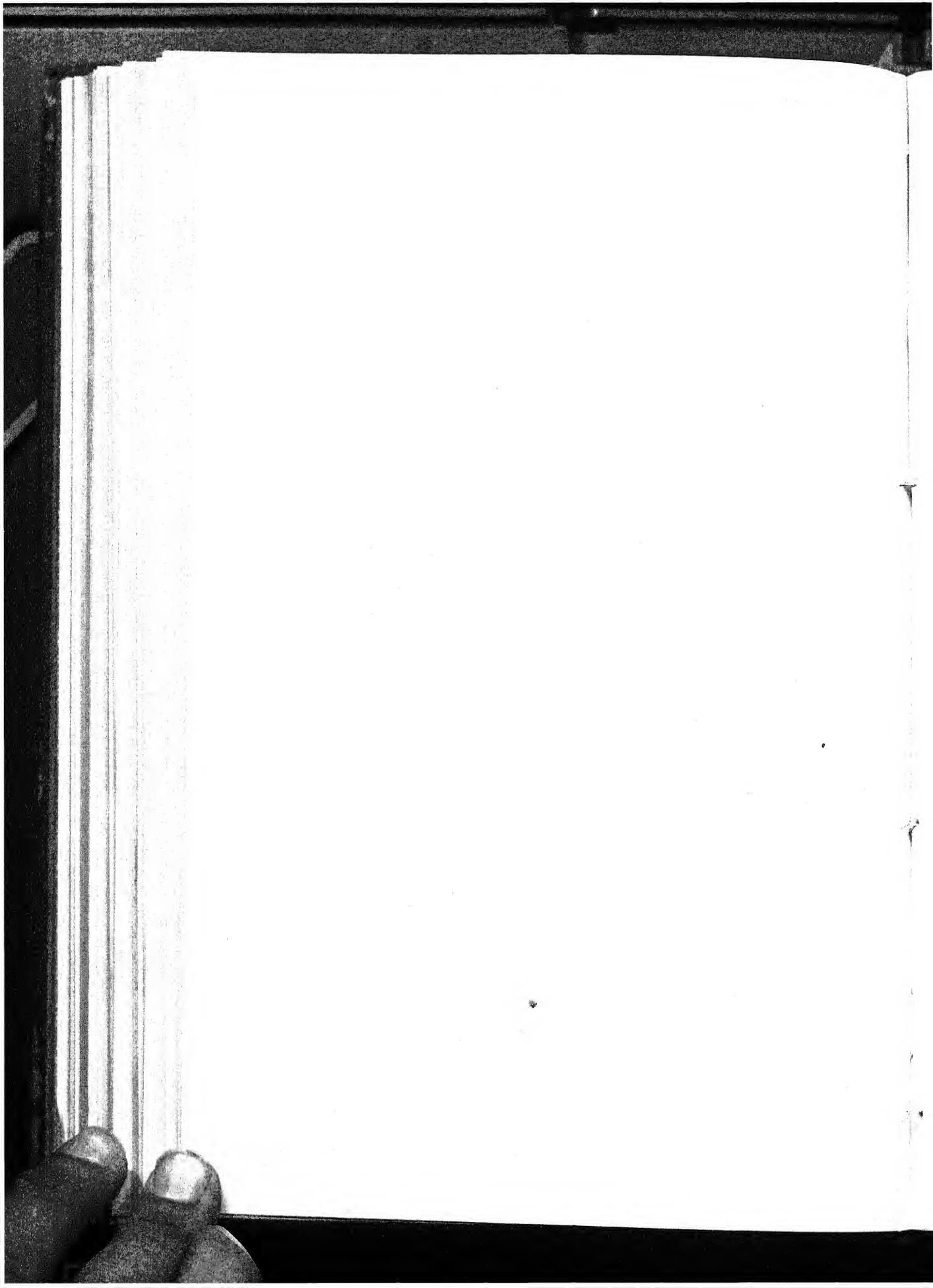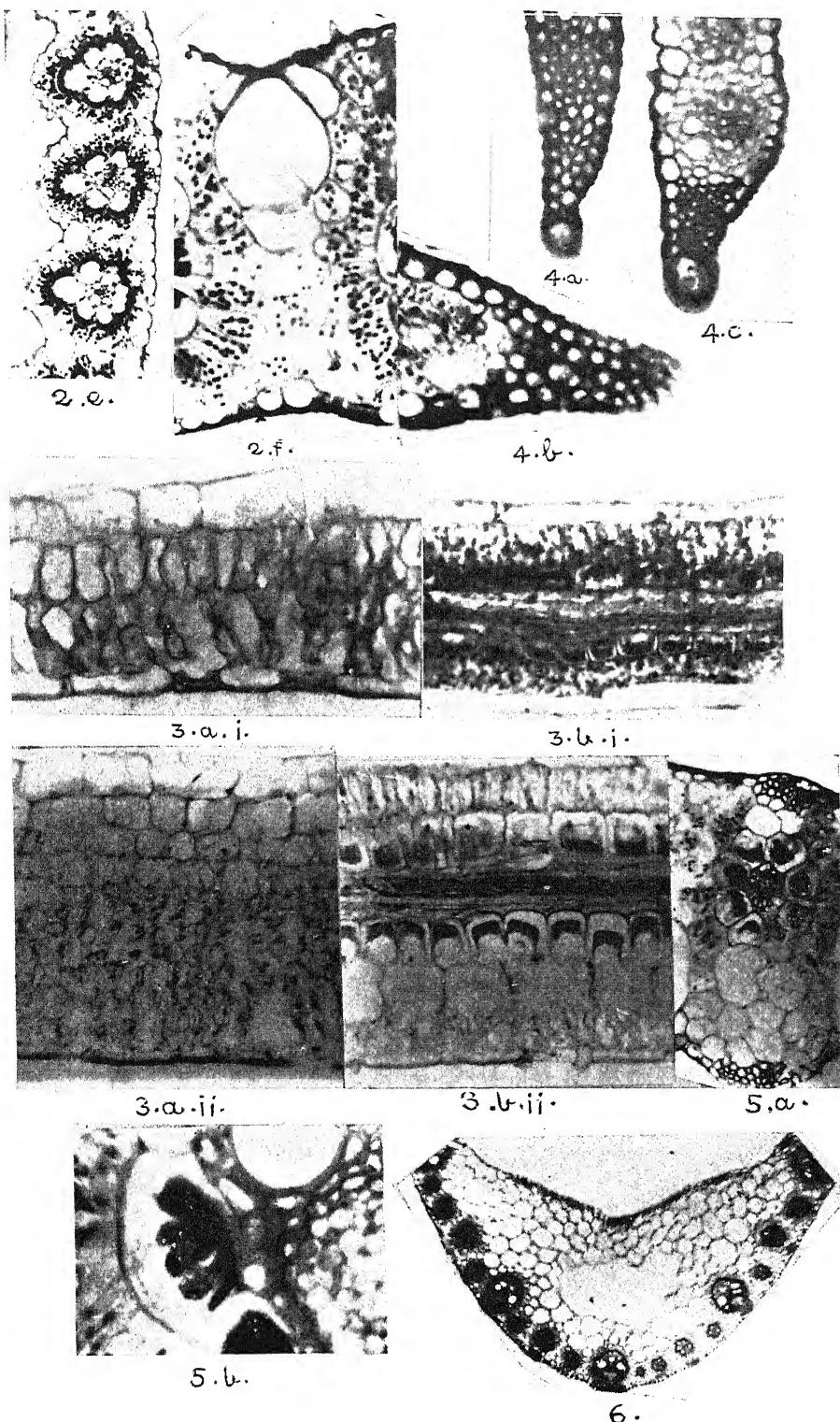
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ANATOMICAL STUDIES IN THE LEAVES OF THE MILLETS
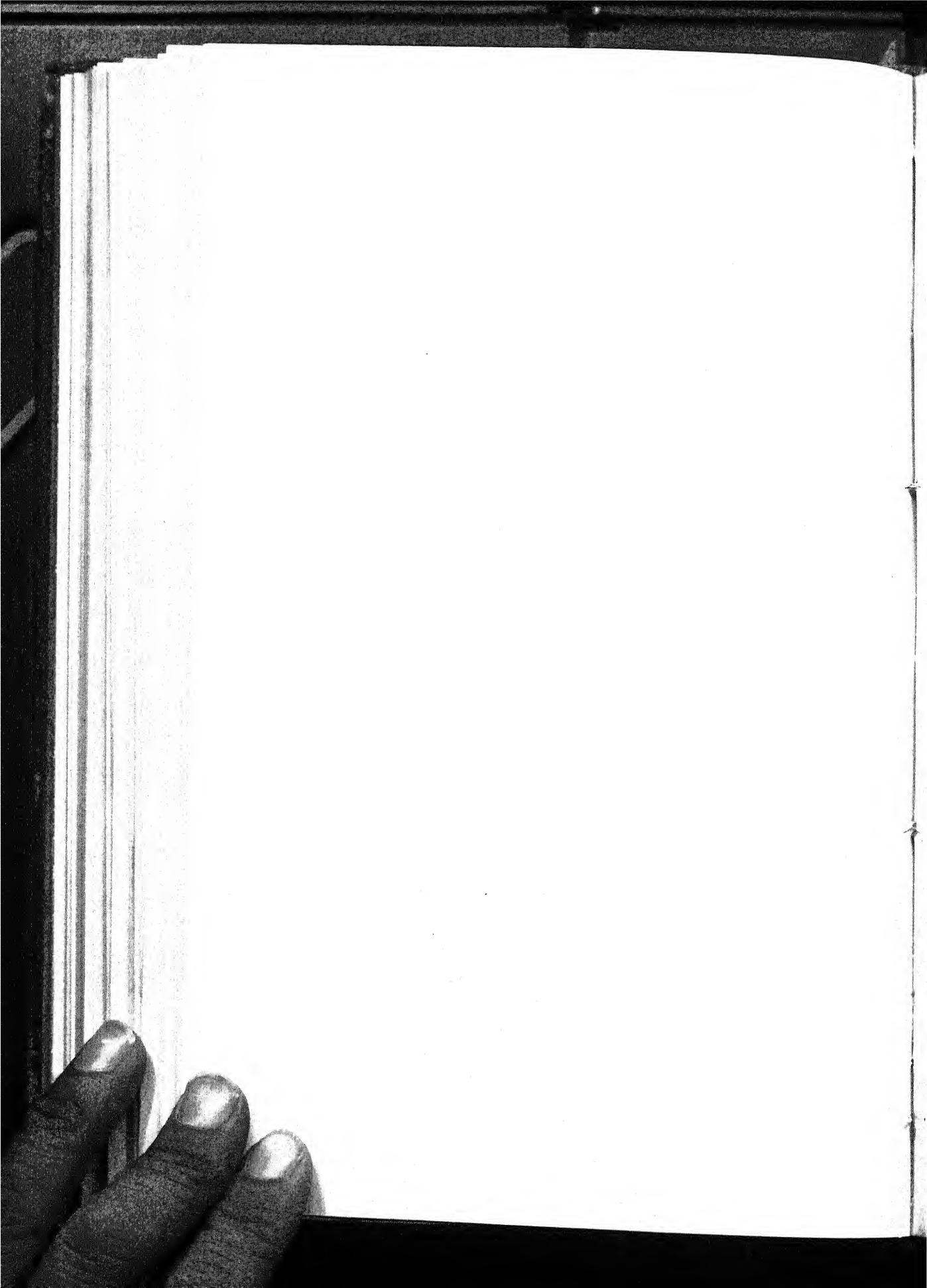
FURTHER OBSERVATIONS ON
VAUCHERIACEAE FROM NORTHERN INDIA

By M. S. Randhawa, I.C.S.

Rœ Bareli

In 1939 the present author[6] described the following six species of *Vaucheria* from the plains of the Punjab and the United Provinces.

V. sessilis, V. geminata, V. uncinata, V. hamata, V. polysperma and *V. amphibia* sp. nov.

It is of interest to note that *V. polysperma* mixed with *V. uncinata* is commonly found on the soft muddy banks of Tons river in tehsil Meja of Allahabad district in the months of January, February and March. *V. polysperma* was also collected from the banks of Chambal river below Pinahat in tehsil Bah, Agra district in January 1941 growing in the form of dark blue-green irregular patches at the sides of small tributary ' Nalas ' which drain into the main stream.

Vaucheria sessilis was also collected from dark cavelike vaults at the sides of Verinag spring in the Kashmir, the source of river Jhelum, on 28-8-1941. Excellent fruiting material of *V. sessilis* forma *orthocarpa* was collected from a tank at Dewaldhar in Kumaon Himalayas. However the most interesting collection made by the present author is that of *V. terrestris* from the Amar Nath Cave in Kashmir, at an altitude of about 12,730 feet above sea-level. In this communication it is also desired to record the collection of *Dichotomosiphon tuberosus* from Burma and various localities in India.

Vaucheria DeCANDOLLE, 1805

1. *Vaucheria sessilis* DeCandolle, forma *orthocarpa* (Reinsch.) Heering = (*V. orthocarpa* Reinsch.) Figs. 1–2.

Filaments 80–105 μ diam., oogonia usually in pairs surrounding an antheridium (Fig. 1) or single (Fig. 2) ovoid or oblong-ovoid, sessile, 66–72 μ broad and 80–95 μ long. Oogonia may be oblique as in typical specimens of *V. sessilis* (Fig. 1) or erect as in *V. orthocarpa* Reinsch (Fig. 2). Presence of oblique and erect oogonia in the same material shows that *V. orthocarpa* Reinsch. is not a valid species, and Heering[4] was right in reducing it to a variety of *V. sessilis*.

Antheridium is straight (Fig. 2), slightly bent (Fig. 1) or circinate, and is borne on a long stalk.

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Habitat.—Free floating in a masonary tank used for feeding a water mill in the estate of R. B. Chiranji Lal Sah at Dewaldhar, 6,500 feet above sea-level, Almora district, Kumaon Himalayas, September 1939.
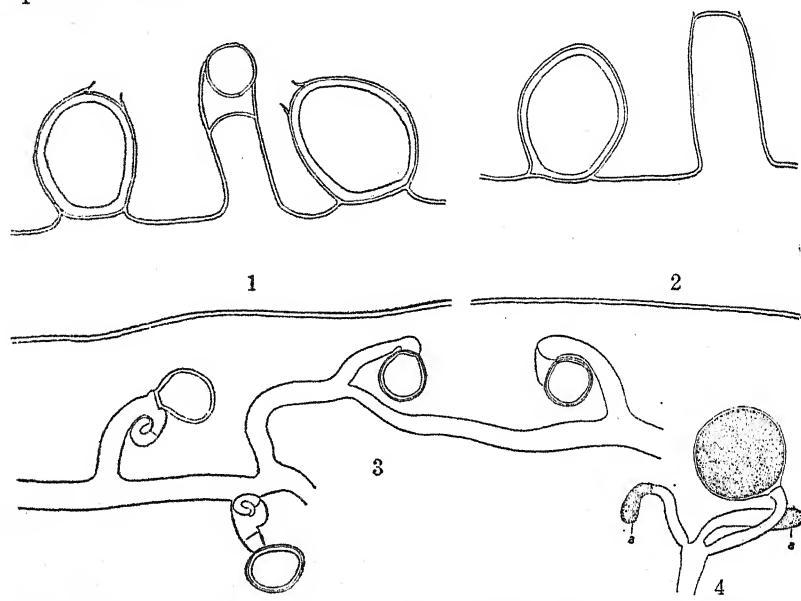
Figs. 1–4.—*Vaucheria sessilis* D.C. forma *orthocarpa* (Reinsch.) Heering.—Fig. 1. A pair of oblique oogonia. Fig. 2. An erect oogonium (Both ×620). *Vaucheria terrestris* (Vauch.) D.C.—Fig. 3. Filaments showing ripe oospores and disposition of sex organs (×120). *Dichotomosiphon tuberosus* (A. Braun) Ernst.—Fig. 4. An oogonium with a ripe oospore surrounded by a pair of antheridia (Indian material from Allahabad)(×120).

2. *Vaucheria terrestris* (Vauch.) *DeCandolle.*—(Fig.3).—Filaments 27–48 μ diam., oogonium solitary, sessile or with a short stalk on the pedicel of antheridium, 90–105 μ × 108–135 μ ; antheridium curved or circinate, terminal, though it sometimes appears to be lateral (Fig. 3) on account of being pushed aside, 10–16 μ diam ; oospore black, globose to plano-convex.

On account of its growing in weak light, filaments were much narrower than the type. The material was abundantly fruiting and showed plenty of oogonia and ripe oospores.

Habitat.—Found growing in the form of yellowish-green or bluish felt-like patches in Amarnath Cave, Kashmir, 12, 730 feet above sea-level on 4th August, 1941. It was growing below the wooden-railing at a distance of about 30 feet from the mouth of the cave on moist soil over which water was trickling from the limestone roof.

DICHOTOMOSIPHON Ernst. 1902

1. *Dichotomosiphon tuberosus* (A. Braun) Ernst (Fig. 4).

Two varieties of this alga were collected, one from Burma which is bigger than the type, the other from various localities in India, which is appreciably smaller.   These are separately described below.

(*A*) *Burman material.*—Filaments 108–130 μ diam., 1–12 cms. long. Oogonia single, terminal, surrounded by pairs of outcurved ; antheridia borne on long stalks.   Oogonia 320–360 μ diam., antheridia 53–63 broad and 180 μ long.

Habitat.—Collected by Dr. S. C. Varma from a fresh-water stream, attached to stones near Maymyo, Burma on 3rd September 1939.

(*B*) *Indian material.*—Filaments dark green in colour, 72–100 μ broad, deeply constricted.   Rhizoids dark or light-brown in colour.

Oogonia, single, terminal, surrounded by pairs of antheridia borne on long stalks (Fig. 4). Oogonia 240–260 μ diam. containing a dark green oospore completely filling the oogonium.

Antheridia cylindrical, curved ; 50–64 μ broad and 120–200 μ long.

Habitat.—This alga was found growing in big masses covering acres of land along the shallow banks of Jumna river near Rajapore, district Allahabad.   From the edge of the water line it grows to a depth of 2–3 feet.   It was also collected from Tons river in Allahabad district in February and March 1940 bearing bumper crops of oogonia which appear to the naked eye like poppy seeds in size.   In February 1941, it was collected by the author in a rapidly flowing tributary of Chambal river, below Pinahat in Agra district, in the form of dark bluish-green cushions along with a species of *Enteromorpha* in sterile condition.

So far as the present author is aware this is the first record of a fertile material of *D. tuberosus* from the United Provinces. Some sterile material of the alga was collected by Mr. Mehr Chand Sethi of Lahore from near Murree, a sample of which was sent by him to the author.

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A CONTRIBUTION TO THE EMBRYOLOGY OF *JUSSIEUA REPENS*, LINN.

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With 26 Figs. in the Text

1. Introduction

THE universal occurrence of the tetranucleate embryo-sac in the Onagraceæ has made this family especially interesting to the student of morphology, the fact being regarded so significant that the only exception, *Trapa*, which has an 8-nucleate embryo-sac (Ishikawa, 1918) has been placed in a separate family, the Hydrocaryaceæ (see Engler, 1936, p. 306). The monosporic tetranucleate embryo-sac in the Onagraceæ was first discovered by Geerts (1908). A résumé of the later work is given by Ishikawa (1918) and Gates (1928). More recently Johansen (1928–34) has published a series of papers on the Onagraceæ in which he has made some valuable additions to our knowledge of the morphology of this family. In 1934 Maheshwari and Gupta published a brief report of their observations on the development of the embryo-sac in *Ludwigia parviflora* and *Jussieua repens*. One of the latest contribution to the embryolo y of the family has been made recently by Beth (1938) who studied the problem of adventive embryony as caused by wounding. The present paper, though complete in itself, is a continuation of the study of *J. repens* initiated by Dr. Maheshwari.

2. The Material and Method

The material was collected by Dr. P. Maheshwari and Mr. B. L. Gupta from places near about Agra and fixed in formalin-acetic-alcohol and Nawaschin's fluid. The sections, cut at 5–8 microns, were stained in Heidenhain's iron-alum hæmatoxylin and Safranin—Fast Green.

3. Ovary and the Ovule

The gynæcium is completely inferior, pentalocular with a vertical row of ovules in each locule, the placentation being axile.

On the ovary a little above its base are seen two one on each side, leaf-like structures in which there is a vascular bundle corresponding to the midrib. During its further course, the bundle branches so that in a transverse section at a higher level three to five bundles may be visible. The trace going to the leaf-like structure originates in the vascular system of the ovary. Johansen

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(1931 *d*) observed such vestigial adventitious leaves on the ovary of *Anogra pallida* and infers that it was leaf-bearing at one time like the ovaries of *Gongylocarpus* and *Burragea*.

Some of the epidermal cells of the ovary enlarge and become elongated to form unicellular hairs. The wall of the ovary has air chambers which become smaller and less numerous towards the top. Bundles of needle-like crystals—raphides—are present in a considerable number of cells which are much larger than the ordinary cells.

The ovules arise as small swellings which exhibit a continued curvature, as they grow, turning through an angle of 270° during their development. Usually they are anatropous though in a very few exceptional cases, the orthotropous form was also observed (Fig. 1). There are two integuments. The outer does not grow on all the sides simultaneously, arising late on the side towards the funicle (Fig. 4) and, even in the older stages is not so well marked on that side, being in close contact with the raphe (Fig. 2).

In the young ovule neither hypostase nor epistase is present but after fertilization the cells of the nucellus lying in the chalazal region acquire a changed appearance, their contents staining more and more densely and the cell walls also becoming somewhat thicker. Older stages show a well-formed and conspicuous hypostase in the chalaza with or without a column extending to the base of the embryo-sac cavity (Fig. 2). In one case the hypostase was found to be fully developed although there was no indication of an embryo-sac having been formed in this ovule, which, however, contained two young embryos (Fig. 26 *a*). Nuclei can clearly be seen in the cells of the hypostase when it is young but, later, a number of granules staining like nucleoli appear and it becomes difficult to distinguish the nuclei. In still older stages the whole cavity of the cell is filled with a mass of some substance which takes an intense and uniform stain. A somewhat similar change is observed in some cells in the base of the raphe. These cells, later, extend from the base to the middle of the raphe and isolated cells may be seen between them and the hypostase indicating that, probably, in the oldest stages, the two may become continuous (Fig. 2). A hypostase extending into the raphe as far as the funiculus has been described in *Epilobium watsoni* var. *franciscanum* (Johansen, 1928). In *Jussieua* some cells resembling those of the raphe, just described, are also found in the wall of the ovary.

It is interesting to note that Johansen (1928), feeling the need of a revision of the earlier theories regarding the presence of the hypostase and its functions in the ovule of the Onagraceæ and particularly those hypotheses advanced concerning the function or functions of the former, studied the different aspects of this problem. He writes, "Comparison of the data regarding the presence or absence of the hypostase with the known habitat of the plant in nature revealed that species growing in the water (*Ludwigia mulertti*), in boggy situations (*Circaea pacifica*), in damp places or near waterfalls (*Epilobium obcordatum*), in places where there is sufficient
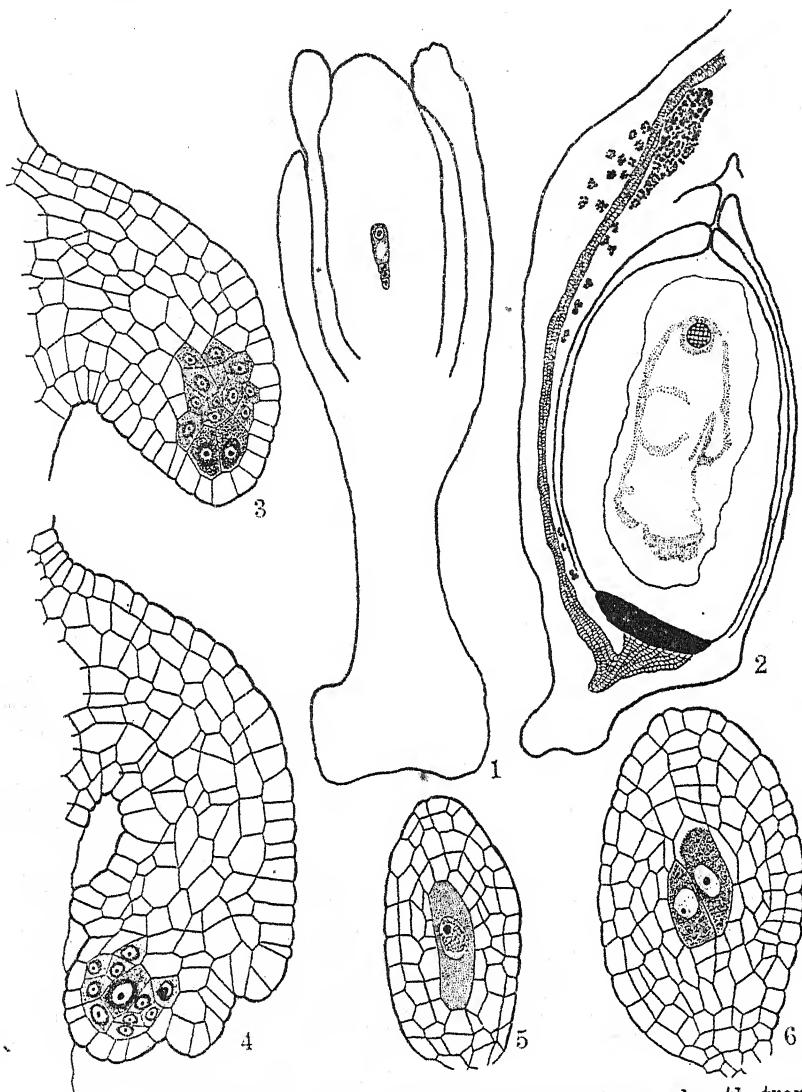
Figs. 1–6.   *Jussieua repens.*—Fig. 1.   L.s. of an abnormal, orthotropous ovule (×159).   Fig. 2.   L.s. of an old ovule showing the hypostase (black). Inside the nucellus are seen the embryo (of which the suspensor is broken off) and the endosperm (dotted) ×48.   Fig. 3.   L.s. of a young ovule showing two cells of a multicellular archesporium growing simultaneously. Fig. 4.   The archesporial cell has divided into the primary wall cell and the megaspore mother cell. Fig. 5. Megaspore mother cell pushed downward by the wall layers. Fig. 6. Two megaspore mother cells in the same nucellus ×470.

moisture present in the soil to keep it from drying out completely during the late summer (*Epilobium minutum*), or have the ovules

enclosed in fleshy berries (most species of *Fuchsia*), lack completely all evidence of a hypostase. On the other hand, species inhabiting regions in which a long summer drought prevails (*e.g.*, the Moja e Desert and lower San Joaquin valley in California) invariably possess well-developed hypostases and, in a few cases, an epistase." He concludes from this that the hypostase and the epistase serve to stabilize the water balance of the resting seed over the long period of dormancy during the hot, dry season. *Jussieua repens* is a remarkable exception to the above observations and conclusion, possessing a fully developed and well-marked hypostase in spite of the fact that it is completely aquatic though it may be found growing on dry mud also when the water has receded.

### 4. ARCHESPORIUM AND THE MEGASPORE MOTHER CELL

The hypodermal cells of the young ovule differ from others in having more conspicuous nuclei and comparatively densely stained cytoplasm in which there is no appreciable vacuolation and thus give the impression of a multicellular archesporium. Usually only one of these cells develops further; in one case two were found growing simultaneously (Fig. 3). Evidently every cell in the hypodermal region is potentially an archesporial initial though usually only one or in a few cases two develop and become recognizable as such.

Two megaspore mother cells in the same nucellus were twice observed (Fig. 6). The occurrence of more than one mother cell has also been reported in *Taraxia ovata* (Johansen, 1931*a*). Ishikawa (1918) once observed three embryo-sacs in an ovule of *J. repens* two of which, he thinks, were produced by the products of one megaspore mother cell and the third by those of another. That usually only one mother cell or embryo-sac develops to maturity seems to be due to the limitations of space and nutrition.

The archesporial initial divides periclinally into the primary wall cell and the megaspore mother cell (Fig. 4), the former dividing further and producing a number of wall layers which bury the megaspore mother cell into the middle of the nucellus. In this position it exhibits a remarkable increase in size and prepares for the reduction divisions (Figs. 5 & 7).

5. THE TETRAD

The megaspore mother cell undergoes reduction divisions producing a linear tetrad of megaspores (Fig. 8). This arrangement is normal for the family though deviations are not unknown. For instance *Zauschneria latifolia*, *Anogra pallida* ((Johansen, 1931*c* and 1931*d*) and *Ludwigia parviflora* (Maheshwari and Gupta, 1934) sometimes show ⊥-shaped tetrads while *Fuchsia* var. " *Diadem* " (Täckholm, 1915) occasionally has the T-type. In *Stenosiphon linifolium*, Johansen (1931*b*) observed that frequently a wall is not organised in the chalazal dyad. A similar condition was once observed in *Jussieua repens* in connection with the micropylar dyad which
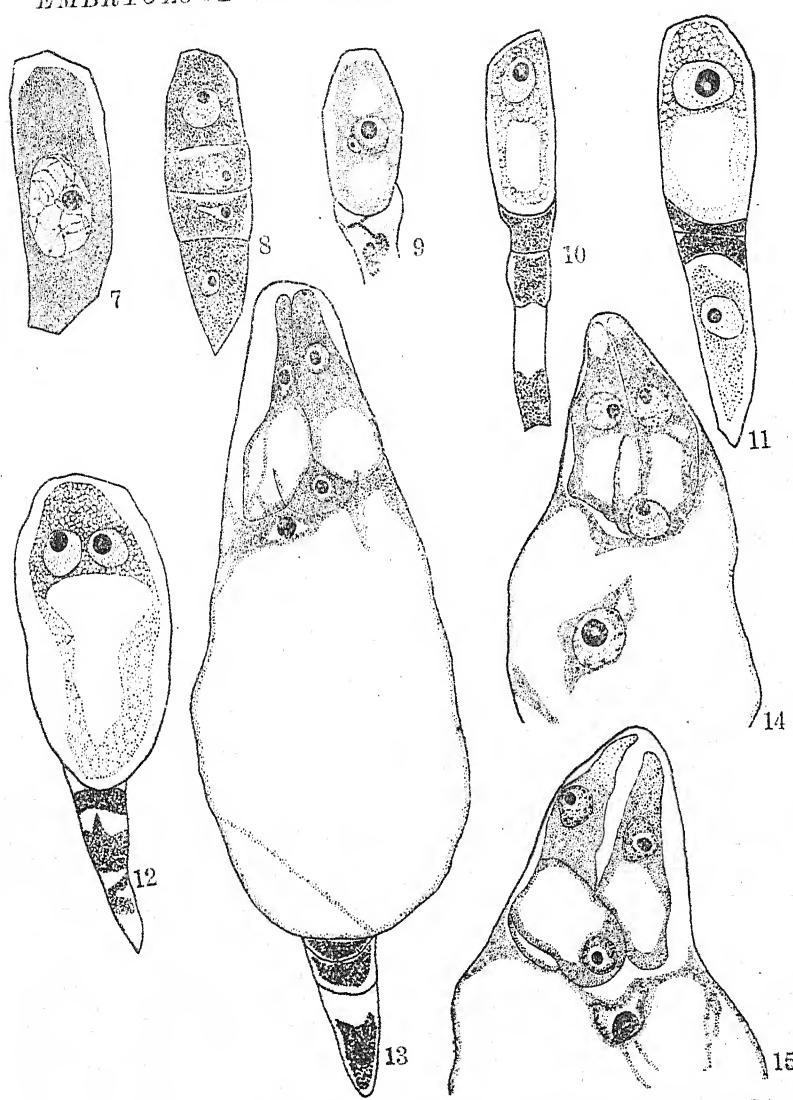
Figs. 7–15. *Jussieua repens.*—Fig. 7. Megaspore mother cell in prophase of reduction division. Fig. 8. Linear tetrad. Fig. 9. An abnormal megaspore with two nuclei one of which is very small; for explanation see text. Fig. 10. Functional megaspore with a single, large vacuole which has pushed the nucleus towards the micropyle. Fig. 11. The two terminal megaspores of a tetrad enlarging simultaneously; the middle ones have degenerated. Fig. 12. Two-nucleate stage of the embryo-sac with the nuclei side by side. Fig. 13. The mature, 4-nucleate embryo-sac. The degenerating megaspores are still seen clearly. Fig. 14. Upper half of an embryo-sac; the synergids have lightly stained tips (reconstructed from two sections). Fig. 15. Embryo-sac with the egg situated obliquely to the longitudinal axis of the embryo-sac. ×945.

showed a small functionless nucleus lying close to the larger nucleus
destined to give rise to the constituents of the embryo-sac (Fig. 9).
This interpretation is not quite certain, however, as the chalazal
megaspores although appearing to be only two, could not be de-
finitely counted. This case is more or less comparable to the one,
reported by Joshi (1939), in *Iphigenia indica* in which the chalazal
megaspore forms the embryo-sac. The development is said
to follow the *Normal*-type, occasionally the *Scilla*-type. In one
case it was found that no wall appeared in the chalazal dyad cell
after the second meiotic division and the nucleus corresponding
to the megaspore next to the functional one was small and likely
to degenerate. From this Joshi concludes that the embryo-sac
in this case would have developed from the cytoplasm of two but
the nucleus of one megaspore and that it would thus be intermediate
between the *Normal*- and the *Scilla*-types. Following this line
of argument, it may be said that the embryo-sac that would have
developed in *J. repens* in the case cited above, would be intermediate
between the *Oenothera*- and the *Allium*-types.

Degenerating tetrads were observed in several ovules in some
of which the degeneration had proceeded so far that there was no
trace of a tetrad having been formed except the presence of a few
densely staining bodies which may be taken to represent the de-
generated megaspores, the nucellus in these ovules being quite
solid and without any indication of embryo-sac formation at all.

Usually the micropylar megaspore develops into the embryo-
sac, the other three degenerating before the functional megaspore
has developed to its fullest extent, though the product of degene-
ration persists for a long time and was mistaken by older workers
for antipodals. The chalazal megaspore is seen to become remark-
ably elongated before degeneration. In some cases the chalazal
(Fig. 11) and in one the megaspore next to the micropylar were
observed to enlarge simultaneously with the micropylar one though
not exactly to the same degree. In one preparation seven ovules
(out of 45) possessed two embryo-sacs each, lying one above the
other, and in some of these it could easily be ascertained that they
had been produced by megaspores belonging to the same tetrad.
As already mentioned, Ishikawa (1918), working on *Jussieua
repens* observed three embryo-sacs in the same nucellus, two of which
he believes to have arisen from megaspores belonging to the same
tetrad. Beth (1938) reports a similar phenomenon in *Oenothera
lamarckiana* in which occasionally two megaspores of a tetrad,
by their concurrent development, may give rise to twin sacs ; in
one case a " Drillingsembryosack " was observed and it is held
more probable that the three megaspores producing this composite
structure came from the same tetrad. It is thus clear that every
one of the four megaspores is potentially functional.

6. The Embryo-sac

The functional megaspore has at first no conspicuous vacuole
and its nucleus is situated in the middle. As development proceeds,

two prominent vacuoles appear, one in the micropylar and the
other in the chalazal end (Fig. 9). The former soon disappears
while the latter increases in size, the nucleus being pushed towards
the micropyle (Figs. 10 and 11). Here it undergoes two succes-
sive divisions producing four nuclei which become organised into
two synergids, one egg and one polar nucleus (Figs. 12 and 13).
Judging from the relative positions of the two nuclei produced by
the first division it appears that this spindle is either transverse
to the longitudinal axis of the embryo-sac or oblique. In the latter
case one nucleus is slightly lower than the other and it may be
inferred that the lower one produces the egg cell and the polar
nucleus and the upper the synergids. But when the spindle is trans-
verse so that the resulting nuclei lie side by side (Fig. 12), it cannot
be said with certainty which of them will produce the synergids and
which the egg cell and the polar nucleus. The binucleate stage
of the embryo-sac of *Ludwigia parviflora* figured by Maheshwari and
Gupta (1934) also seems to have been derived from an oblique
division spindle. The organisation of the mature embryo-sac, how-
ever, is normal in both *L. parviflora* and *J. repens* though Johansen
(1932) attributes to the transverse division spindle the erratic
organisation of many embryo-sacs in *Gayophytum ramosissinum.*

The synergids have a swollen base occupied by a large vacuole
while the nucleus is seen in the neck-like micropylar portion. Both
the indentations and the filiform apparatus are absent (Fig. 13).
Only one case was observed in which there was some indication
of the indentations. One or two preparations were encountered
in which the tips of the synergids were almost stainless (Fig. 14).

The synergids often exhibit irregularities of structure and
position. The neck-like micropylar portion may be short and
broad or pointed or it may be entirely absent. In one case a
synergid possessed no vacuole and was triangular in shape.

The egg cell, in a longitudinal section, is pyriform or oblong
in outline, the micropylar end being slightly narrower than or as
broad as the basal. The nucleus lies in the basal portion embed-
ded in a small mass of cytoplasm, the rest of the cell being occu-
pied by a large vacuole (Fig. 14). Sometimes the outline may
be irregular (Fig. 19). Usually the nucleus possesses a single pro-
minent nucleolus but, in rare cases, it may be binucleolate. In
one case the egg was found to be without the characteristic vacu-
ole; its micropylar end was broad, the cell tapering to a point to-
wards the chalaza. In a few cases the egg was lying obliquely to
the longitudinal axis of the embryo-sac (Figs. 15 and 19).

Of all the four nuclei of the embryo-sac, the polar is the most
conspicuous. The outline of this nucleus, as seen in a section, is
somewhat circular but irregularities are common (Fig. 15). It
is embedded in an irregular mass of cytoplasm which is not bounded
by a membrane (Fig. 14). The nucleolus is large and very often
contains one or more crystalline structures (Fig. 16). In *Oeno-
thera* (Ishikawa, 1918) also the polar nucleus is very prominent and

its nucleolus always contains one or several vacuoles which are replaced by a crystalline structure. In *Hartmannia tetraptera* and *Taraxia ovata* (Johansen, 1929 and 1931 *a*), on the other hand, the polar nucleus is frequently non-nucleolate. In *Clarkia elegans* (Johansen, 1930) the polar nucleus is binucleolate while in *Zauschneria latifolia* (Johansen, 1931 *c*) it (the polar nucleus) is often missing. In *Hartmannia tetraptera* (Johansen, 1929) when it is present, it is often amœboid in shape. In *Anogra pa lida.* (Johansen, 1931 *d*) the polar nucleus divides repeatedly by budding and may produce as many as 140 daughter nuclei.

7. ABNORMAL EMBRYO-SACS

A number of abnormal embryo-sacs exhibiting irregular organisation or abnormalities in the number of nuclei were observed and are described below :—

1. Although the embryo-sac was fairly old, none of the four nuclei was organised into a cell, all of them lying embedded in irregular cytoplasmic masses.

2. In Fig. 16 one of the synergids is seen to be devoid of its nucleus but near the egg nucleus there is observed another nucleus which is smaller and slightly curved. This may be the missing synergid nucleus though it does not appear so owing to its curvature and small size. It is also possible that the small nucleus belongs to the egg and is degenerating while the larger may be regarded as the displaced synergid nucleus for it resembles the other synergid nucleus very closely. A pollen tube was not met with in this ovule.

3. Fig. 17 shows a 5-nucleate embryo-sac in which the nuclei are not organized into cells and lie embedded in a common mass of cytoplasm. From their relative positions and sizes, however, two of them may reasonably be regarded as corresponding to the egg and the polar nuclei and two to the synergid nuclei. The fifth is seen near one of the latter and it seems likely that it was produced by budding from the nucleus near which it is seen. This conclusion is supported by the fact that the other synergid nucleus is also seen to be in the process of producing a bud which has not yet separated from the mother nucleus. Johansen (1931 *d*) has reported a similar division of one of the synergid nuclei in *Anogra pallida* in which the number of daughter nuclei may reach twenty due to repeated budding.

4. The embryo-sac shown in Figs. 18 *a* and 18 *b* is seen to possess three extra nuclei. There was no trace of a pollen tube having entered the ovule and the extra nuclei cannot, therefore, be regarded as belonging to the male gametophyte. These might have been produced by the division of some of the four nuclei that formed the normal embryo-sac or it may be that what appears to be a single embryo-sac has been produced by the fusion of the products of two developing megaspores.*

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* In this connection see also Beth (1938).
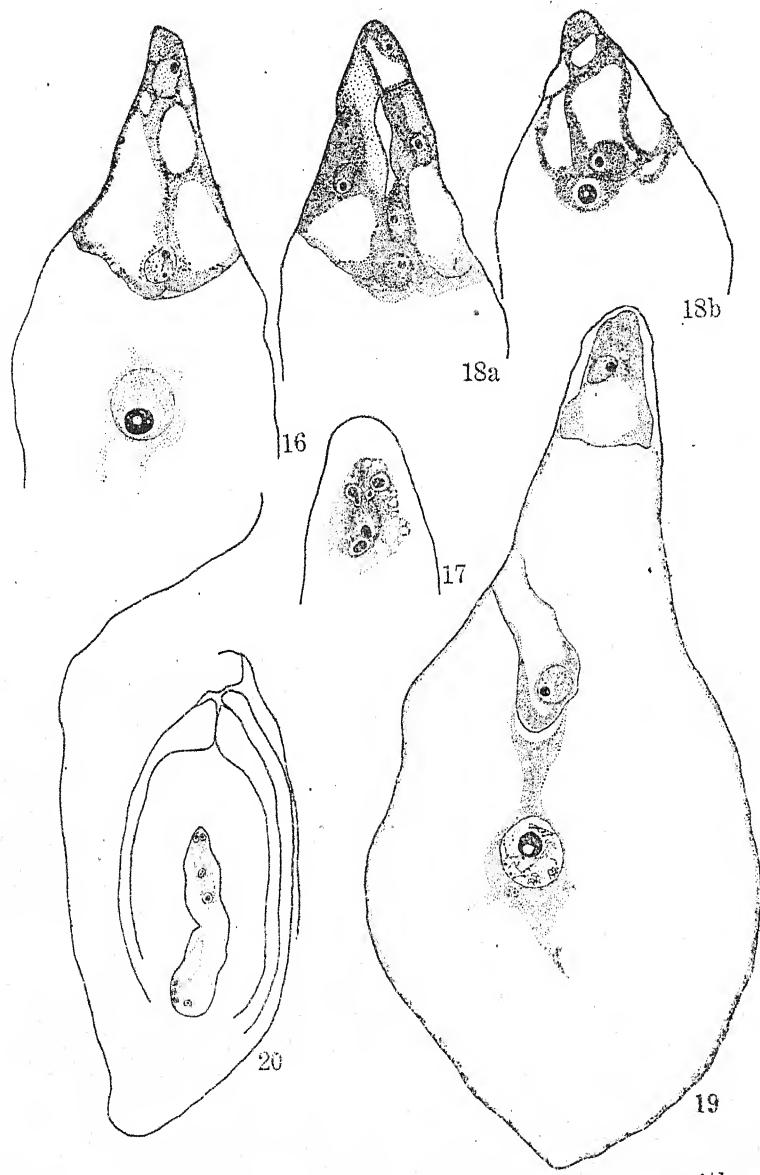
Figs. 16–20. *Jussieua repens.*—Fig. 16. Embryo-sac with a small nucleus situated near that of the egg. The nucleus of one of the synergids is not seen (reconstructed). Fig. 17. Embryo-sac with one synergid nucleus divided into two while the other is in the budding condition. Fig. 18. *a* & *b*. Successive sections of an embryo-sac with 7 nuclei; for explanation see text. Fig. 19. Three-nucleate embryo-sac with a single synergid. ×756. Fig. 20. Two 4-nucleate embryo-sacs in the same nucellus appearing like a single, 8-nucleate embryo-sac due to their cavities having become continuous (reconstructed) ×127.

5. Fig. 19 shows a large, 3-nucleate embryo-sac in which the synergid mother nucleus has evidently failed to divide and has itself organised into a single synergid of comparatively larger size. Both the egg cell and the polar nucleus are sit ated much lower down than usual, the former being attached to the lateral wall of the embryo-sac. A 3-nucleate embryo-sac of similar origin has also been reported in *Hartmannia tetraptera* (Johansen, 1929).

6. As already mentioned, twin embryo-sacs were observed in several ovules. The micropylar sac in all these cases was fully developed and mature while the chalazal exhibited a retarded development. In such rare cases when the latter possessed all the four nuclei they showed none of the characteristic organization of a mature embryo-sac though, in one case, from their relative positions two of them seemed to correspond to the synergids and the other two to the egg and the polar nucleus.

In one ovule the cavities of the two sacs—both of which were at the 4-nucleate stage—were found to be continuous. Such a case may be mistaken, at the first glance, for a single normal, eight-nucleate embryo-sac because the nuclei of the lower sac, due to incomplete organisation, can be regarded as the three antipodals and the lower polar nucleus of the *Normal*-type of embryo-sac (Fig. 20).

8. FERTILIZATION

The pollen tube enters the ovule through the micropyle (Fig. 21). The male nucleus comes in contact with the female and exerts some pressure so that the sides in contact become flattened. Dissolution of the two membranes begins at this place and the two nucleoli now lie within a common nuclear membrane. Their fusion is, however, considerably delayed; in one case in which the endosperm consisted of about 15 nuclei, they were still seen to be separate and distinct (Fig. 21). The second male nucleus was observed lying near the polar nucleus. A third nucleus besides the two male nuclei was seen in one or two embryo-sacs; this may be the tube nucleus.

9. EMBRYOGENY

The embryogeny is normal (see Souèges, 1920). The first division of the zygote is transverse, producing a basal (towards the micropyle) and an apical cell (Fig. 22). The latter th n divide, longitudinally (F g. 23). followed by a transverse division of the former. Fig. 24 shows the 4-celled embryo of which the two apical cells produce the cotyledons, plumule and the hypocotyl, the next is the hypophysis cell while the basal forms the suspensor. Fig. 25 shows an old embryo with cotyledons well developed.

In one preparation two embryos were seen lying side by side in the same nucellus (Figs. 26 *a* and 26 *b*). This nucellus was also peculiar in showing no cavity representing the embryo-sac. Extending from the tip of one of the embryos to that of the nucellus was a longitudinal passage in which could be seen the remains of the

pollen tube. Most probably an embryo-sac never organised in this ovule and the embryos have been produced by nucellar cells after or without fusion with the male nuclei. The mere presence of the remains of a pollen tube cannot lead to any definite conclusion, while a chromosome count could not be made.

With the growth of the embryo, the tissues of the ovule undergo some remarkable changes. The development of the hypostase has already been described. The nucellus becomes reduced to a few cells almost entirely restricted to the micropylar and the chalazal portions. The middle portion of each integument consis s of two layers of cells. The cells constituting the inner layer of the inner integument, have their inner (those towards the nucellus) walls thickened while those of the outer layer become elongated, their walls acquiring pitted thickening. The cell; of the inner layer of the outer integument contain an abundance of
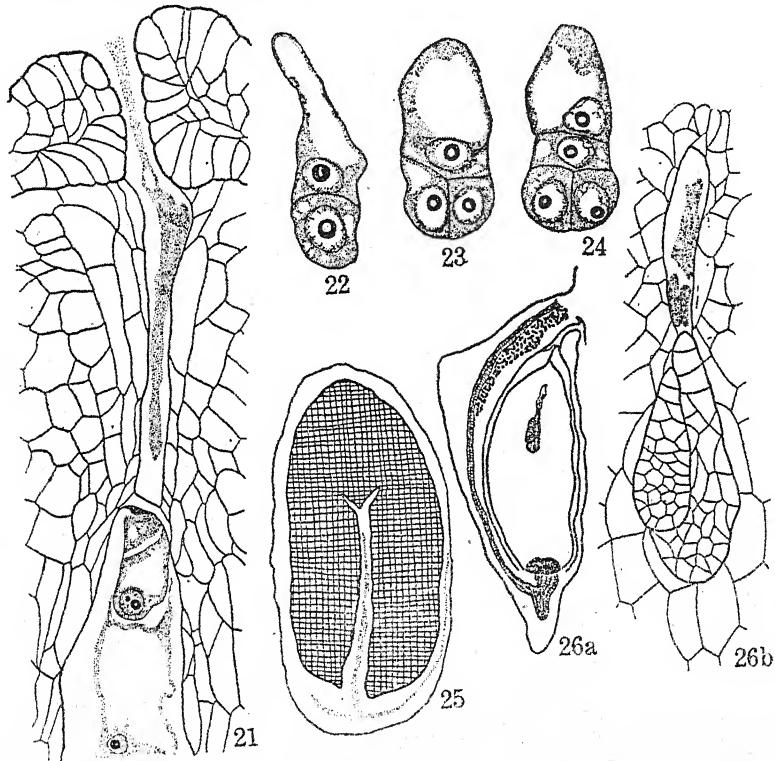
Figs. 21–26. *Jussieua repens.*—Fig. 21. L.s. of an ovule showing the entry of the pollen tube through the micropyle. The zygote st.ll shows the two gamet'c nucleoli. ×376. Fig. 22. Two-celled embryo. Fig. 23. Same, terminal cell d'v'ded vert cally. F g. 24. Four-celled embryo. ×480. Fig. 25. Embryo w th well-developed cotyledons (d agrammat c) ×38. Fig. 26 *a.* L.s. of an ovule with two embryos in the same nucellus ×38. Fig. 26 *b.* A port.on of the same magnified ×216.

crystals and also have their radial and inner walls specially thickened while those of the outer layer do not show any special modification.   The micropyle becomes closed by a plug-like structure.

10. ENDOSPERM

The oldest endosperm observed consisted of about 900 free nuclei embedded in a parietal layer of cytoplasm.   It could not, however, be ascertained whether it becomes cellular later on or remains in the free nuclear condition till the end.† The nuclei are mostly uni-, bi- or tri-nucleolate ; some may possess even six nucleoli while in one the number was ten.   Nuclei with more than three nucleoli are mostly found in the chalazal region where also the cytoplasm is comparatively denser.   In one preparation the nucleoli in every nucleus were seen connected with one another by fine threads, producing, in some cases, the appearance of a network with large granules at the angles of the meshes.

There is some evidence favouring the conclusion that at least some of the endosperm nuclei divide amitotically.   A budding or dumb-bell-shaped nucleolus is occasionally seen but in one case a nucleus also, containing four large and two minute nucleoli, was seen to be bilobed.   Occasional amitotic divisions of endosperm nuclei have been reported in *Zauschneria latifolia* (Johansen, 1931 *c*). It cannot be said whether in *Jussieua repens* all the nuclei divide amitotically though a mitosis was never observed.

11. DISCUSSION

The occurrence of the 4-nucleate embryo-sac outside the family Onagraceæ, as far as our present knowledge goes, is not common. Ishikawa (1918) gave a list of such cases some of which have been definitely proved to be wrong.   Most of the remaining cases also, included in the list given below, are now regarded as doubtful (see Maheshwari, 1937 and 1941) and a reinvestigation of all of them is necessary.

Balanophoraceæ :
Helosis guayanensis (Chodat   See Fagerlind (1938)
and Bernard, 1900)

Urticaceæ :
Elastostemma acuminatum   A four-nucleate embryo-sac was
(Strasburger, 1910)   observed in a few cases only

† At a stage when the embryo is as old as that shown in Fig. 25, the nucellus is reduced to 2 or 3 layers of cells. The cells of the innermost layer are no ably different from those of the other layers. Also a degenerating tissue which, sometimes, can be seen clearly to be cellular, is present between the cotyledons. If this tissue and the layer of cells just referred to are the remains of the endosperm, it would obviously mean that the latter becomes cellular in its older stages. The lack of a sufficiently close series of stages in the endosperm development, does not, however, warrant a definite conclusion.

Podostemonaceæ :
  Podostemon subulatus
    (Magnus, 1913)
  Hydrobium (= *Zeylandium*)
    olivaceum (Magnus, 1913)
  Farmeria metzgerioides
    (Magnus, 1913)
  Weddelina squamulosa
    (Chiarugi, 1933)

These were reported to possess the *Podostemon*-type of embryo-sac. *Podostemon ceratophyllum* (Hammond, 1937) has been found to have an embryo-sac which has been classified under the *Allium*-type by Maheshwari (1941) who now thinks that it is doubtful if the *Podostemon*-type exists at all

Euphorbiaceæ :
  Glochidion (Arnoldi, 1912)
  Ceramanthus (Arnoldi, 1912)
  Codiæum (Arnoldi, 1912)

See Lundberg (1931) on *Codiæum variegatum*, and Maheshwari and Chowdhry (1937) on *Phyllanthus* (= *Ceramanthus*)

Commelinaceæ :
  Commelinantia Pringlei
    (Parks, 1935)
  C. anomala (Parks, 1935)

See Maheshwari and Singh (1934) and criticism by Maheshwari (1937)

Liliaceæ :
  Clintonia borealis
    (Smith, 1910)

See alternative interpretation by Maheshwari (1937)

Orchidaceæ :
  Gastrodia elata (Kusano, 1915)
  Cypripedium spectabile (Pace, 1907)
  C. parviflorum (Pace, 1907)
  C. pubescens (Pace, 1907)
  C. candidum (Pace, 1907)

See Maheshwari (1937) and also Carlson (1941) on *Cypripedium parviflorum*

  Gyrostachys cernua (Pace, 1914)
  G. gracilis (Pace, 1914)
  Bletia shepherdi (Sharp, 1912)

A four-nucleate embryo-sac was seen in some cases only.

A genuine 4-nucleate embryo-sac outside the Onagraceæ is present only in *Plumbagella micrantha* (Boyes, 1939) but this differs from the ordinary 4-nucleate embryo-sac in being tetrasporic and acquiring the 4-nucleate condition secondarily. Of the four nuclei resulting from the two successive divisions of the megaspore mother cell nucleus, one moves to the micropylar end and divides into two while the other three move to the chalazal end and fuse together to form a single triploid nucleus which divides into two.

12. Summary

1. The ovary is inferior and pentalocular the placentation being axile. It bears two reduced, leaf-like structures, one on each side, a little above its base.

2. The ovules are usually anatropous and during their development turn through an angle of about 270°. A well-developed

and conspicuous hypostase appears with the development of the embryo.

3. Though the usual number of megaspore mother cells in an ovule is one, sometimes two are also found. Some preparations give an indication of a multicellular archesporium although eventually only one or two cells enlarge further. There is a linear tetrad of megaspores of which the micropylar functions as a rule although occasionally other megaspores especially the chalazal may also enlarge and simulate the functional one.

4. The embryo-sac is monosporic and 4-nucleate. Synergid indentations and filiform apparatus are absent. The polar nucleus is very prominent. In a few ovules there was no sign of the embryo-sac which probably degenerated at a very young stage.

5. The pollen tube enters the ovule through the micropyle. The male and the female nuclei are similar. The fusion of their nucleo i is considerably delayed.

6. Two embryos were once found in the same nucellus although an embryo-sac was not recognisable.

7. The oldest endosperm observed consisted of at least 900 free nuclei but it could not be ascertained if it remains free nuclear till the end. Some of the endosperm nuclei divide a mitotically.

8. Some abnormal embryo-sacs including three-, five and seven-nucleate ones have been described. Twin embryo-sacs formed by two megaspores were also found in several ovules.

9. Besides the Onagraceæ, a 4-nucleate embryo-sac occurs only in *Plumbagella*. All other cases are doubtful and need reinvestigation.

13. ACKNOWLEDGEMENT

It gives me great pleasure to acknowledge my indebtedness to Dr. P. Mahes wari for his sympathetic g idance and for his kindness in giving over to me his preparations of *Jussieua repens* upon which this study is based.

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GASTEROMYCETES OF N. W. HIMALAYAS II

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Calvatia Fr.

THE genus *Calvatia* was proposed by Fries but it was emended and made known to science by Morgan (1890). It differes from *Lycoperdon* in the dehiscence of its peridium: in *Lycoperdon* the peridium opens by a definite mouth while in *Calvatia* it breaks up into pieces and falls away exposing the gleba. The genus includes all of the larger species formerly included among the *Lycoperdons*.

The species described here undoubtedly belong to the genus *Calvatia* on account of their very large size but differ, however, in the peridium not irregularly scaling away at maturity but opening by a definite mouth. As the distinguishing character between the two genera is not always constant, so in most cases one has to rely on individual judgement in referring a species to one or the other genus. This explains why *Calvatia* species have so often been referred to the genus *Lycoperdon*.

15. *Calvatia saccata* (Fr.) Lloyd, Myc. Writ. I: 166, 1904.

Syn. *Lycoperdon saccatum* Fries, Syst. Myc. III: 35. Arnigadh, Mussoorie, 5500 ft., in groups on the ground (W. Gollan) Baramula, Kashmir (A. N. Fotidar). Nov. 1940.

Reported by Hennings (1901) as *Lycoperdon saccatum* and according to him the spores are globose, $3-4 \cdot 5\mu$, yellow olive, punctate; the capillitium threads yellow brown, $3 \cdot 5-4 \cdot 5\mu$ thick.

The Kashmir specimen is preserved in the Herb. Ind. Orient. New Delhi. It has a very well developed sterile base, the peridium opening by a definite mouth; capillitium brown, of unseptate freely branched threads with pitted walls; spores globose, verrucose, $3 \cdot 5-4 \cdot 65 \mu$ in diameter.

16. *Lycoperdon piriforme* (Schæff.) *Pers., Syn. Meth. Fungi* 148, 1801.

Plants obovoid or pyriform, $1 \cdot 3$ cm. broad and $1 \cdot 5-4$ cm. high, the base tapering, stem-like, attached by abundant mycelial threads. Exoperidium breaking into distinct irregular furfuraceous black scales and more or less persistent black warts; endoperidium membranous, pale brown or dark brown, opening by an irregular torn aperture; sub-gleba white, compact, noncellular occupying the stem-like base. In Kashmir specimens the

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sub-gleba consists of distinct cells and is not so well developed as in Naggar plants.

Gleba olivaceous brown; capillitium branched, very rarely septate, rounded at the septa, olivaceous, tapering at the ends; spores globose, 3–4·45 μ in diameter with a large central vacuole; epispore pale olive-brown, smooth.

Naggar, Kulu, 5,500 ft., on the ground. *Leg.* S. Ahmad, Sonamarg, Kashmir, 9000 ft., *Leg.* R.R. Stwart.

The specimens from Naggar agree in every respect with Kambly and Lee's species (1936) but differ, however, from Cunningham's plant (1927) in which the sterile base is occupied by large pallid or yellowish cells more than 2 mm. in size and the spores are verrucose. The Kashmir plant resembles the latter in the sterile base.

Examination of the material of this species in the Herb. Crypt. Ind. Orient. New Delhi, shows, that the species is peculiar in showing two distinct forms with regard to the variation in the sub-gleba. In the American specimens from Ohio (No. 02901), the sub-gleba consists of distinct concolorous cells, while in specimens from S. India (No. 65), it is white and not at all cellular but of a homogeneous tissue.

17. *Lycoperdon umbrinum Pers.*, *Syn. Fung.* 147, 1801.

Plants up to 3·2 cm. in diameter and 4·5 cm. in height, sub-globose, obovate or turbinate with a rooting base. Exoperidium furfuraceous in the form of small fugacious granules; endoperdium umber-brown, papyraceous, opening by an apical irregularly torn aperture ; sub-gleba paler, of small cells occupying the lower third or less ; diaphragm absent.

Gleba purplish brown; capillitium threads Ausique Brown (Ridgway), freely branched, unseptate, smooth, 2·8–4·2 μ in diameter ; spores globose, 4·46–5·58 μ in diameter ; epispore Ausique Brown (Ridgway), verrucose mixed with fallen pedicels.

Khanag, Kulu, 8,000 ft., solitary on the ground, *Leg.* S. Ahmad.

This species is very close to *Lycoperdon atropurpureum* from which it is distinguished only by its exoperidium. The species is described by Coker and Couch (1928) as having capilitium threads more or less uneven, with numerous or scattered distinct pits in the wall but in the writer's plant the threads have perfectly even surface.

A few specimens of this species were sent to Dr. G. H. Cunningham for identification and he remarks " Lycoperdon sp. undescribed, I think. It has reticulated spores, a feature I have not seen in any Lycoperdon or Bovista ". The reticulated appearance of the spores is due to the spines which on soaking in water appear as dark striations in the thick hyaline material forming the wall (*cf.* Coker and Couch, p. 77).

Lycoperdon Berkeleyi de Toni in Sacc. Syll. Fung. VII: 124, 1888, Syn. *L. delicatum* Berk. (non Berk. and Curt.) Hook. Jour. Bot. VI: 172, 1854.

This species collected from Simla is preserved in the Herbarium of the Forest Research Institute, Dehra Dun. According to Lloyd (1905a) it is only a depressed globose form of *L. Umbrinum Pers.*

Lycoperdon elongatum Berk. in Hook. Jour. Bot. VI: 171, 1854.

Arnigadh, Mussoorie, 5,500 ft. on the ground (W. Gollan). According to Lloyd (1905) this is only a subcylindric form of *Lycoperdon atropurpureum* with a small head. Hennings (1901) described, the gleba umber-brown, spores globose, purple-brown, verrucose, 5–6 μ in diameter, capillitium threads branched 1–1·5 μ thick, reddish-brown.

18. *Lycoperdon perlatum* Pers., *Syn. Fung.* 145, 1801. *Syn. L. gemmatum* Fr. Syst. Myc. III: 36, 1829.

Plants turbinate, up to 3·2 cm. in diameter and 3·8 cm. in height. Exoperidium of thick pyramidal stout spines, each surrounded by a ring of smaller spines which on falling give a reticulated appearance to peridium, the spines towards the base are smaller and more scattered, dark-brown in colour; endoperidium bay-brown or at times yellowish, membranous, opening by an indefinite torn apical aperture. Sub-gleba of large cells occupying the stem-like base.

Gleba brownish; capillitium threads olivaceous, branched unseptate, 3·75 μ in diameter; spores globose, 3·75–4·5 μ in diameter, verrucose.

Mussoorie ; Dalhousie.—on the ground among moss. *Leg.* S. Ahmad.

Kaghan Valley (Specimens in Herb. of the Forest Research Institute, Dehra Dun).

The species is easily recognised by the arrangement of spines and by the reticulated surface of the peridium in the old specimens.

19. *Lycoperdon trachyspora* (Lloyd). S. Ahmad comb. nov. Syn. *Bovistella trachyspora* Lloyd Myc. Writ. II: 287, 1906.

Plants globose or sub-globose up to 1·8 cm. in diameter, attached to the ground by a well developed mycelial base. Exoperidium furfuraceous or of very small granules, whitish or nearly black in colour, falling off at maturity to leave the endoperidium perfectly smooth ; endoperidium thin papery, yellowish or dark-brown, opening by a large apical torn aperture; sub-gleba absent.

Gleba olivaceous changing to dark-brown; capillitium of long, branched and very rarely septate threads, up to 4·5 μ in diameter branches narrow tapering, yellowish or pale-brown; spores globose,

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4·5–5·25 μ in diameter, verrucose; pedicellate, pedicel slender, nearly hyaline, tapering, up to 10·5 μ in length.

Dalhousie; Chamba; Mussoorie, growing among moss. *Leg.* S. Ahmad.

The species has been transferred from *Bovistella* to *Lycoperdon* because of its capillitium with long, branched and intertwined threads, typical of the genus. It is closely related to *L. echinella* (Lloyd) S. Ahmad in general appearance, but it differs from it in having markedly rough spores.

The spores and capillitium have been compared with the type received from J. A. Stevenson, Washington, D.C. and are the same.

20. *Lycoperdon Mundkuri* S. Ahmad Sp. Nov.

Plants 2·1 cm. in diameter and 2 cm. in height, obovoid with small stem-like base attached by abundant mycelial threads. Exoperidium of more or less persistent minute spines arranged in fasciculate groups, giving the endoperidium a pitted appearance. The spines more prominent and numerous near the mouth, but smaller and scattered towards the base, appearing granular to the naked eye, brownish in colour. Endoperidium membranous, pale-coloured opening by an apical torn aperture. Sub-gleba scanty, of small cells occupying the very small stem-like base.

Gleba brownish; columella prominent: capillitium threads of long, branched, unseptate, 4·5 μ diameter, very rarely pitted; spores oval 3–3·2 ×4. 5–6 μ; epispore yellowish, smooth ; pedicellate, pedicel straight or curved, nearly hyaline, 10·5–18 μ in length.

Khanag, Kulu, 8,000 ft., on the ground. *Leg.* S. Ahmad. Type,—In the Writer's Herbarium.

The species is characterised by the exoperidium, presence of columella, oval and pedicellate spores and a scanty sterile base. It is very closely related to *L. pedicellatum* from which it is distinguished by the exoperidium and oval elliptical spores with smaller pedicels.

Lycoperdon Mundkuri S. Ahmad Sp. Nov.

Plantae 2·1 cm. diametro 2 cm. altitudine, obovatæ, fundaments parvo, stirpi simili, multis mycelialibus filis, afflcts; exoperidis minutis spinis fere persistentibus, fasciculatis catervis compositis, facto ita ut peridium foveatum sit; sporæ ad os prominentiores et mumerosiores mut, sed ad fundamentum mut minores et diffusæ; ocula tantum cum visæ sunt, granosæ et fuscæ. Endoperidii mumbrana pallidissima, ad os exapice scissum aperta. Sub-gleba exigna, parvis cellis facta, in fundamento stirpi simili collorata.

Gleba fusca; columella prominens; fila capillaria longa, ramis inseptatis, 4·5 μ latis, rarissimis foveis; sporæ ellipticæ 3·0–3·2 ×4·5–6 μ, epispora fulvus et levis; pedicillatum, pedicellum directum vel flectum, pæne hyalinum, 10·5–18·0. μ longum.

Hab.—In terra ad Khanag, Kulu, 8,000 pedes alt; *Leg.* S. Ahmad.

21. *Lycoperdon hiemale* Bull. Champ. P. 143 Syn. L.
pratense Pers. *L. depressum* Bonord.

Chamba, solitary on the ground. *Leg.* S. Ahmad.

The specimens agree well with the plant already described from the Panjab plains by the writer (*J. Ind. Bot. Soc.*, Vol. XVIII, 1940). The sterile base is separated from the gleba by a distinct diaphragm; capillitium hyaline septate, sparsely branched.

22. *Lycoperdon Wrightii Berk. and Curt. Grevillea* II: 50, 1873.

Plants globose or depressed globose, up to 2 cm. in diameter and 1·3 cm. in height, sessile or with a short stem firmly attached to the soil by numerous mycelial threads. Exoperidium of small spines arranged in fasciculate groups, each group falling off singly. Endoperidium Tawny-Olive (Ridgway), membranous, opening by an indefinite apical mouth slightly furfuraceous. Sub-gleba of small cells occupying the lower third of the plant separated from the gleba by a well developed diaphragm.

Gleba light dark-olive (Ridgway), capillitium hyaline, branched, rarely septate, septa very numerous, $3·6–6·45\,\mu$ in diameter; spores globose $3·6–4·5\,\mu$ in diameter, epispore pale-yellow, finely verrucose.

Mussoorie, 7,000 ft., in grassy places. *Leg.* S. Ahmad.

According to Coker and Couch (1928) this species is in reality a synonym of *L. Curtisii* Berk. as shown by the co-type in the Curtis Herbarium (Wright, No. 7; Curtis, No. 5633).

23. *Lycoperdon marginatum Vitt. Monogr. Lyc.* 1842. Syn.

L. Cruciatum Rostk. in Sturm, Deuts. Fl. III: 19, 1844.

Botanic Garden, Saharanpur (W. Gollan).

Dehra Dun (Herb. of the For. Res. Institute, Dehra Dun).

The species has almost the external characters of L. *Wrightii* Berk. and Curt. and when immature, it is difficult to tell them apart. The plants of this species are usually more than 2 cm. in diameter and the cortex flakes off in very large pieces, whereas in *L. Wrightii* the plants are smaller (less than 2 cm.) and the cortex-spines usually fall singly.

The record of this species based on the determination of Hennings (1901) is doubtful. As Hennings himself remarks " die-exemplare sind sämmtlich unreif, daher nicht sicher bestimmbar." Nothing can be said about Dehra Dun plant preserved in the Herb. For. Res. Inst. as it has not been examined by the writer.

24. *Lycoperdon pusillum Batsch Elench.* II: 288.

Dehra Dun, on the ground (Preserved in the Herb. Cryp.
Ind. Orient., New Delhi No. 464).

A very common and a very variable species already described
from the plains. Coker and Couch (1928) remark " it is a puzzling
species due to variation in spores " but state " the capillitium is
pitted and this character is uniform for all collections ". The
writer, however, finds that the capillitium also varies; in the Panjab
plant it is always smooth with even walls, whereas, in the Hima-
layan collection (No. 464) it has a beautifully pitted surface.

There are three other species of *Lycoperdon* described by
Berkeley from India, *viz., microspermum, xanthospermum* and
emodense which are doubtfully distinct from *L. pusillum.*

According to Berkeley (1851) *L. microspermum* has " all the
characters of *L. pusillum* but the diameter of the spores is not
above half as large." Lloyd on the other hand studied the type
specimens at Kew and failed to find any difference in the spore-
size of the two, and for him *L. microspermum* is the same as
L. pusillum. The spore-measurements from specimens of *L. pusillum*
and *L. microspermum* in the Herb. Crypt. Ind. Orient., New Delhi,
also confirm Lloyd's observation.

L. pusillum Batsch. No. 644 from Dehra Dun.—Spores
globose, apiculate, smooth, $3 \cdot 6$–$4 \cdot 8$ μ in diam., mean being 4 μ.

L. microspermum Berk. No. 297 from Gauhati, Assam.–Spores
globose rarely apiculate, sligthly echinulate, $3 \cdot 6$–$4 \cdot 5$ μ in diameter,
mean being $3 \cdot 9$ μ.

There is another collection from Shillong, Assam (No. 298)
labelled *L. microspermum* Berk. It differs from the one from Gau-
hati, Assam (No. 297) in the spore-characters. The spores are
globose, pedicellate, smooth, $3 \cdot 7$–$5 \cdot 6$ μ in diameter, mean being
$4 \cdot 6\mu$. This suggests *L. xanthospermum* Berk. instead of *L. micro-
spermum.* The difference between the two according to the original
description is only the pedicellate spores of the former. *L. xantho-
spermum* is closely related to *L. pusillum* and *L. emodense* and
according to Berkeley (1854) it differs from these only in the nature
of the outer peridium and the pedicellate spores.

With regard to *L. emodense* Berkeley remarks " very distinct
from *L. microspermum* of which it has somewhat the appearance in
its larger spores." The material of this species is not available but
the description suggests that there is hardly any difference
between it and *L. pusillum* and *L. microspermum*, so out of the four
species only two, *viz., L. pusillum* and *L. xanthospermum* should be
retained, at least for the present while the other two, *viz., L. emodense*
and *L. microspermum*, should be reduced to synonymy of
L. pusillum.

25. *Lycoperdon polymorphum* Vitt. Monogr. Lycop. 183.

Plants up to 3·5 cm. in diameter and up to 5·5 cm. in height, sub-globose or turbinate, contracted into a stalk-like base. Exoperidium of evanescent layer which tends to break into minute scales; endoperidium thin papery, opening by an apical orifice; sub-gleba of minute cells concolorous with the gleba and occupying the stem-like base.

Gleba dull-olivaceous to brown; capillitium brownish, branched unseptate 3·6–4·8 μ in diameter, branches with pointed ends spores globose, 3·5–4·45 μ in diameter, epispore pale-yellow, smooth or faintly verrucose.

Mussoorie; Sonamarg, Kashmir.—Solitary or in groups on the ground rich in humus. *Leg.* S. Ahmad.

26. *Lycoperdon nigrum* Lloyd. *Myc. Writ.* I: L. 2 : 2, May, 1904.

Plants up to 3·3 cm. in diameter and 4·5 cm. in height, turbinate with a very well developed stem-like sterile base. Exoperidium of numerous black spines which impart a black colour to the entire plant ; endoperidium membranous, opening by an apical orifice ; sub-gleba not cellular, but of a homogeneous tissue occupying the stem-like base.

Gleba olivaceous ; capillitium of long branched, unseptate coloured threads with pointed ends; spores globose, 3·6–4·5 μ in diameter, epispore pale-yellow, verrucose.

Mussoorie.—Solitary on the ground rich in humus. *Leg.* S. Ahmad.

According to Lloyd "*L. nigrum* can perhaps be best described as a black form of *L. polymorphum* with the same spores, capillitium and compact sterile base; it differs only in the notably black peridium ". Butler and Bisby (1931) state under this species " We are unable to interpret the name applied by Lloyd. Perhaps he meant *L. nigrescens* Pers." This is not in accordance with facts as *L. nigrescens* is a black form of *L. perlatum* Pers.

FAMILY: SCLERODERMATACEÆ

27. *Scleroderma bovista Fries, Syst. Myc.* II: 48, 1829. Syn. *S. texense* Berk. ; *S. columnare* Berk. and Broome.

Plants depressed-globose up to 3 cm. in diameter and 1·8 cm. in height, plicate below with a short sessile rooting base firmly attached to the soil by numerous separate threads. Peridium thin, firm, areolate near the apex but somewhat furfuraceous at the base, dehiscing by irregular rupture of the apical portion, yellow or dark-brown.

Gleba Saccardo's Olive (Ridgway), spores globose, 10·5–14·5 μ in diameter and including the reticulum which is upto 2·5 μ, epispore dark-brown, strongly reticulate.

Naggar, Kulu, 5,500 ft.; Dalhousie, on the ground. *Leg.* S. Ahmad.

Scleroderma columnare Berk. and Br. recorded by Butler and Bisby (1931) as a distinct species is for Lloyd (1918, p. 759) a stipitate form of *S. cepa* but for Cunningham (1931) it as a form of *S. bovista*. There are no specimens available, otherwise it is not difficult to decide, as *S. bovista* differs from *S. cepa* in having reticulate spores.

28. *Scleroderma dictyosporum* Patouill.
Bull. Soc. Myc. de France 133, 1896.

Plants globose up to 1·7 cm. in diameter and 1·2 cm. in height, with a well developed mycelial base prolonged into a stalk. Peridium coriaceous with indistinct areolations visible under a lens. Gleba yellowish, with numerous yellow thread ; spores globose, 8·2–9·95 μ in diameter ; epispore yellow, strongly reticulate with the reticulum up to 2·5 μ high.

Dehra Dun, on the ground. *Leg.* E. J. Butler.

A small species distinguished from others by its size, yellowish gleba and beautifully reticualted spores up to 10 μ in diameter. It is known from the original collection and is abundantly represented in the Herb. Cryp. Orient., New Delhi.

29. *Scleroderma verrucosum* (Vaill.) *Pers. Syn. Meth.* 154, 1801. Syn. *S. nitidum* Lloyd.

Plants globose or sub-globose up to 3·5 cm. in diameter and 2·5 cm. in height with a very well developed mycelial base, very often produced into a long stalk. Sometimes several plants grow from a common base. Peridium thin, fragile, Light-Buff to Cinnamon-Buff (Ridgway), areolate, dehiscing by a small torn irregular mouth. Gleba Sepia (Ridgway); spores globose, 9–11·5 μ in diameter; epispore pale ferruginous, verrucose, spines pointed up to 1·45 μ in length.

Dalhousie; Mussoorie; Dehra Dun, gregarius on the ground. *Leg.* S. Ahmad.

Scleroderma caespitosum Lloyd recorded by Butler and Bisby (1931) as a new form of *S. verrucosum* (teste Lloyd) is according to Cunningham (1931, p. 283) a stipitate form of *S. flavidum*. The specimens (No. 305) in the Herb. Crpt. Ind. Orient., New Delhi, determined by Lloyd as *S. cæspitosum* have definitely reticulate spores and probably belong to *S. comlumnare*—a form of *S. bovista*.

According to Lloyd (1918, p. 759) *S. nitidum* described by Berkeley from India is only a stipitate form of *S. verrucosum*.

30. *Scleroderma aurantium Pers. Syn. Fung.* 153, 1801.

Arnigadh, Mussoorie, on the ground (W. Gollan).

A collection from Mussoorie by William Gollan was referred to *Scleroderma vulgare* Hornem. by P. Hennings (1901). The same has been listed by Butler and Bisby (1931) under *S. aurantium* Pers. The record of *S. aurantium* based on this determination and on the determination of Lloyd' (No. 680 in the Herb. Crypt. Ind. Orient., New Delhi) appears to be very doubtful. These specimens (No. 680) have verrucose spores different from the markedly reticulate spores of *S. aurantium*. Some authors like Lloyd (1905 *c*) and Massee (1889) have described verrucose spores for this species, but it is evidently a mistake.

It is now believed that *S. vulgare* (Hornem) Fr. consists of two species, *S. aurantium* and *S. cepa*. *S. aurantium* Pers. is recognised by its verrucose and usually distinctly warted surface, thick peridium which turns pink when freshly cut, white tramal plates and strongly reticulate spores. *S. cepa* is distinguished from *S. aurantium* in having strongly spinulose spores, not at all reticulate, and the tramal plates heavily encrusted with yellow crystals.

FAMILY : SPHAEROBOLACEAE

31. *Sphaerobolus stellatus* Tode, *Fung. Meckl. I:* 143? 1790.

Chamba, on dung cakes. *Leg.* S. Ahmad.

The species has already been described from the Panjab plains (1940 *Ind. Bot. Soc. Journal*) and the present collection closely agrees with it.

FAMILY : NIDULARIACEÆ

32. *Cyathus Montagnei* Tul. *Monogr. Nidul.* in *Ann. Science Nat.* 79, **1844.**

Dehra Dun, on old baskets.

The species has not so far been recorded from India and according to Lloyd (1906) " It is only known from Brazil ". It is marked in colour and habitat, growing scattered on rough bark to which it is attached by a pad of mycelium. It is distinguished from *C. striatus** in having ferruginous peridium and more elliptic spores.

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* This species (*C. striatus*) has not so far been reported from India and Lloyd (1906) remarks "It occurs only as far as I know in Europe " and Lloyd (1907) also says that Ceylon specimen of Petch is only a form of *Cyathus striatus*. Dr. S. R. Bose points out in correspondence that it is very common in Calcutta.

33. *Cyathus Poeppigii* Tul. *Monogr. Nidul.* in *Ann. Science Nat.* 1844.

Botanic Garden, Saharanpur, in groups on the ground and on charred wood. *Leg.* W. Gollan.

This species differs from *C. limbatus* Tul. collected from Royal Bot. Garden, Calcutta, in having larger spores.

34. *Cyathus stercoreus* (Schw.) de Toni, *Syll. Fung. VII;* 40, 1888.

Chamba, on dung-cakes. *Leg.* S. Ahmad.

A very common species inhabiting manure heaps and dung-cakes. It has already been reported from the Panjab plains. It is distinguished from other species with smooth cups by the outer periodiole-wall having coloured fibrils. Lloyd (1906) points out that as in all other large-spored species the peridioles in this are also frequently sterile.

35. *Crucibulum vulgare* Tul. *Ann. Sci. Nat. III:* 90, 1844.

Plants sub-globose or short cylindrical, sessile up to 1 cm. in height and 0·95 cm. in diameter, the outer surface covered with a brownish tomentum; the inner surface gray, smooth, shining. Peridiole white, up to 2 cm. in diameter, circular, flattened, attached to the cup by a cord-like simple funiculus. Spores smooth, hyaline, ellipsoid, varying greatly in size, $4·8$–$7·5 \times 6·8$–$10·6 \, \mu$.

Dalhousie-Chamba Road, on dead branches. *Leg.* S. Ahmad.

Kambly and Lee (1936) propose a new combination, *Crucibulum Lævis* (Dc.) for it.

FAMILY : PHALLACEÆ

36. *Ithyphallus impudicus* (Pers.) Fischer, *Versuch einer systemt. Ubersicht über die bisher bekannten Phalloideen* 43, 1886.

It is represented by a beautiful specimen in the Herb. Ind. Orient. collected by E. J. Butler from Achibal, Kashmir, growing on the ground.

This species belongs to the section " Reticulati " of the genus *Ithyphallus.* It differes from other species included in this section in having a white pileus and a white receptacle.

FAMILY : SECOTIACEÆ

37. *Gyrophragmium delilei* Mont. *Fl. Alg. I:* 369.

Sonamarg, Kashmir, 9,000 ft., on the ground. *Leg.* R. R. Stewart.

The specimens collected by Dr. Stewart are preserved in the New York Botanic Garden. As remarked by Murrill (1924). " It (*G. delilei*) is very peculiar in appearance, suggesting a double-decked mushroom, the lower part expanding like the flower and

bearing at its centre a small cup supported on a stalk of its own." The plant usually grows in sandy places and its occurence in Kashmir away from its original home, the " sand dunes " on the Mediterranean coasts, is really very remarkable.

ACKNOWLEDGMENTS

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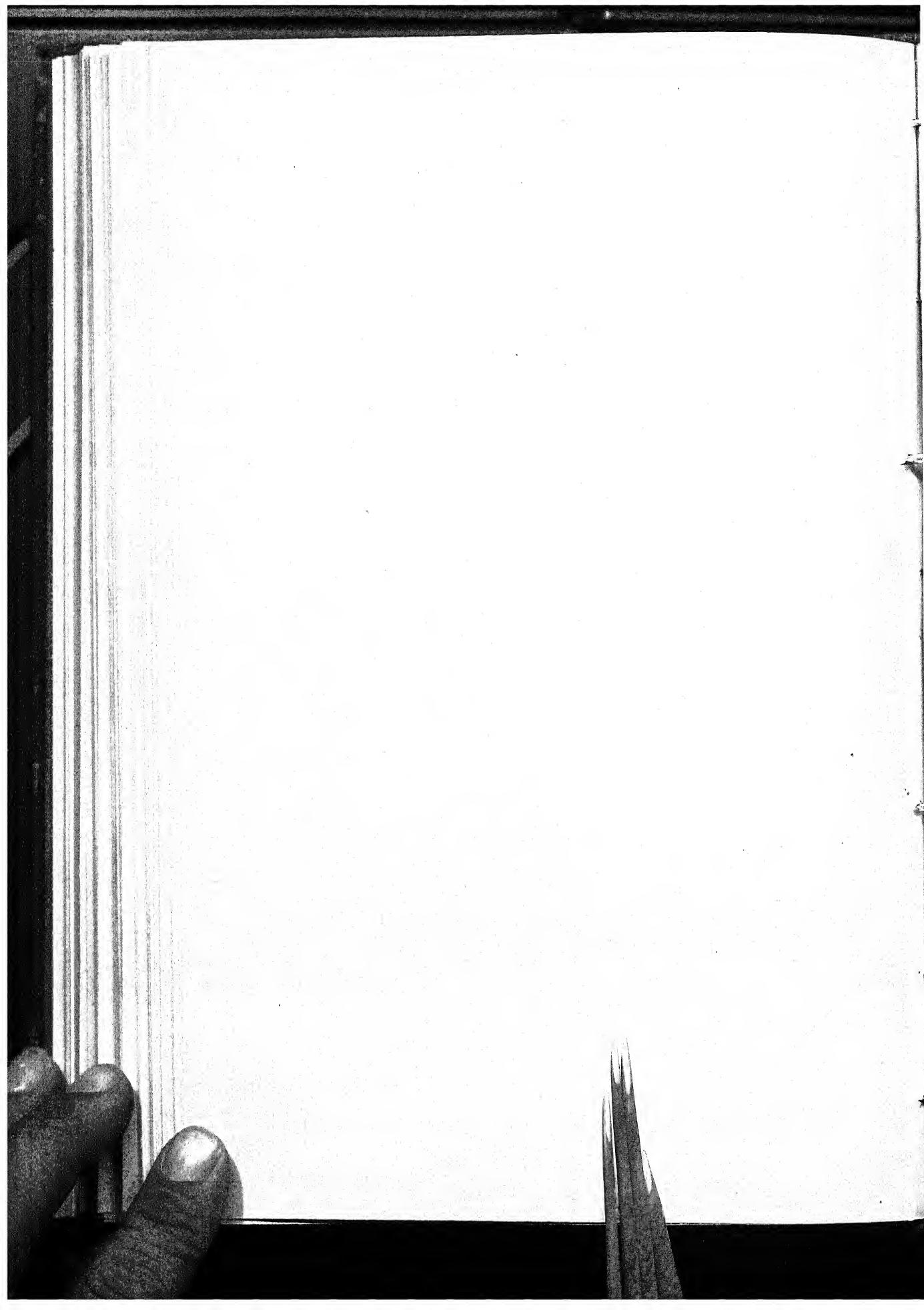
A CONTRIBUTION TO THE LIFE HISTORY OF *BLUMEA LACINIATA* L.

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THE family Compositæ contains about one-tenth of the total number of flowering plants of the world. According to Chatterji[4] it is the second family containing the largest number of species in India. The plants grow under varied edaphic and climatic conditions.

Since the beginning of this century a considerable amount of work has been done on the morphology and embryology of plants belonging to this family, an account of which has been given by Schnarf[19], and recently by Bhargava[3]. Since then a number of important contributions have appeared of which mention may be made of Popham's[16] work on *Galinsoga ciliata*, Cooper's[6] investigations on *Erechtites hieracifolia*, Dianowa and his associates'[8] work on *Parthenium argentatum* and *P. incanum* and the work on *Scorzonera tau-saghys* by Poddubnaja Arnoldi and her co-workers.[17]

In India, Bhargava[3] was the first to work on the morphology of Compositæ. He studied *Eclipta erecta*. This was followed by contributions by the present writer on *Carthamus tinctorious*[1] and *Tridax procumbens*[2]. Datta[7] in 1939 recorded briefly his observations on the development of the female gametophyte in *Launea asplenifolia*, *Blumea laciniata* and *Mikania cordifolia*. This was followed by a brief account of the mega-gametophyte development in two species of *Launea* by Venkateswarlu[23]. Recently Raghavan and Venkatasubban[18] have studied the cytology of *Tridax procumbens*.

MATERIAL AND METHODS

The material for this investigation was obtained from plants growing as weeds in the University college compound. Flowerheads in various stages of development were trimmed and cut into pieces before fixation. As a rule, fixation was always done in the field between 12 noon and 4 P.M., and an exhaust pump was always used to ensure proper penetration of the fixing fluid. Allen's modified Bouin's fluid and Navaschin's fluid were used for fixation. Dehydration, clearing and embedding were done in the customary way. The materials were cut 8 to 16 microns thick depending on the stage required for study. Heidenhain's iron-alum hæmatoxylin was used for staining. Some preparations were counterstained with orange G.

6

INVESTIGATION

(1) *Organogeny.*—In the initial stages of the development of the florets the thalamus is convex in outline and is closely surrounded by the overlapping bracts. The flower primordia appear on this as small papillate protuberances. Very soon the sides grow up and become differentiated as petals (Text-fig. 1). The sepal primordia appear next followed immediately by those of the stamens (Text-figs. 2, 3 and 4). The sepal primordia grow rapidly and become transformed into the pappus. Adopting Small's[20] classification, we can describe the pappus as setose plumate. It is composed of three to four layers of cells which are
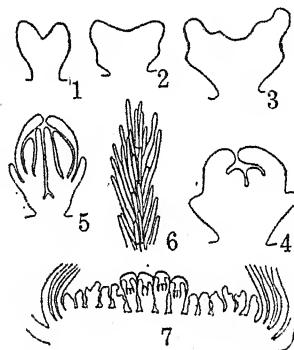
Text-figs. 1–7. *Blumea laciniata.*—Figs. 1–5. Stages in the development of the disc florets. Fig. 6. The upper part of pappus showing the arrangement of cells. Fig. 7. Section through a flower-head showing the earlier development of the central florets. Figs. 1–4 (×85); Fig. 5 (×30); Fig. 6 (×113); Fig. 7 (×28).

arranged in a characteristic manner as shown in Text-fig. 6. The last floral whorl to appear is the gynæcium composed of two carpels which originate close to the centre of the thalamus and on the inner side of the stamens (Text-fig. 5). They grow upwards and fuse above to form the solid style and bifurcate at the top to give rise to the bifid stigma. The central region of the floral axis from the sides of which the carpels develop forms the ovarian chamber. A nectary is present in the form of a ring at the base of the style.

The ray florets are pistillate, while the disc florets are hermaphrodite. As a rule the development of the florets is centrifugal, but in some preparations the central florets have been observed to develop first (Text fig. 7).

The development of the floral organs in *Blumea laciniata* thus appears to differ from that observed by Martin[13] in *Aster,* Bhargava[3] in *Eclipta* and by the present writer[1] in *Carthamus.*

(2) *The development of the pollen grains.*—The anther in the early stages of its development is four lobed in cross section and is

composed of cells which are all alike. The origin of the archesporial cells could not be made out distinctly. The microspore mother cells when first noted appear to be separated from the epidermis by four layers of cells of which the innermost layer forms the tapetum. At prophase the nucleus shows a fine reticulum composed of delicate threads (Text-fig. 8). These soon contract at one side of the nuclear cavity enclosing the nucleolus in its meshes (Text-fig. 9). The contracted knot next opens out and passes through the usual stages of meiotic division before the bivalent chromosomes are organised. At diakinesis, a side by side association of the univalents is most commonly seen (Text-fig. 10). At this stage the pollen mother cells round off and lie free inside the anther cavity. The first division is normal. On the completion of this division the protoplast passes through an interkinetic stage. The second division very soon
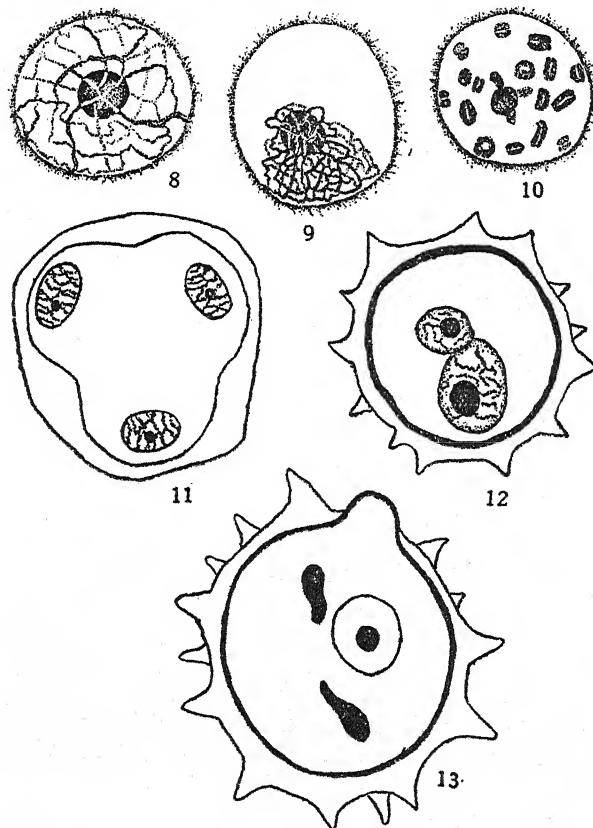

Text-figs. 8–13. *Blumea laciniata.*—Fig. 8. Nucleus in early prophase. Fig. 9. Synizesis. Fig. 10. Diakinesis. Fig. 11. Beginning of cytokinesis. Fig. 12. Binucleate pollen grain. Fig. 13. Pollen grain with the vegetative and generative nuclei (×1650).

6a

follows. The spindles are arranged either parallel or at right angles to each other, resulting in the formation of isobilateral or tetrahedral tetrads. Cytokinesis takes place by furrows which start from the periphery, gradually cut inwards and finally meet at the centre (Text-fig. 11). The four young microspores when first formed lie enclosed by mucilaginous pellicles which disorganise later. The young microspores when first formed are uninucleate and somewhat oval in shape. They soon increase in size, round up and develop the outer and the inner coats. The exine shows the presence of a large number of spines and the intine also becomes thick, as observed by Bhargava[3] in *Eclipta erecta*. The nucleus of the microspore divides to give rise to a generative and vegetative nucleus of which the former is smaller than the latter (Text-fig. 12). The generative nucleus soon divides and produces two nuclei which are somewhat elongated (Text-fig. 13). A similar nuclear condition of the mature pollen grains has been noted by many investigators working in other Compositæ.

The mature pollen grains are echinate and the spines have broad bases. Four germ-pores are present which appear to be situated each in the centre of a furrow. Spines are absent at the intracolpar region.

(3) *The tapetal cells*.—The tapetum is a single layer of well-defined cells which are at first uninucleate (Text-fig. 14). Their nuclei divide mitotically when the pollen mother cells are in synizesis and binucleate cells are thus formed (Text-figs. 15 and 16). Further division of some of these nuclei results in multinucleate cells (Text-figs. 18 and 19). This multinucleate condition is, however, not long maintained, as the nuclei come to lie very close to one another, fuse and then divide again. Large metaphasic spindles with the chromosomes aligned at the equatorial region and occupying the entire length of the cell were seen in many preparations (Text-fig. 21). On completion of divison the tapetal cells become binucleate again but the nuclei are very large and elongated (Text-figs. 17 and 23). In other instances, the nuclei of some of the tapetal cells instead of fusing, divide separately, so that a number of chromosome plates are formed (Text-fig. 20). The close association of the groups of chromosomes indicates the probability of the organisation of only two daughter nuclei after the division is complete. When the microspores have been formed, the tapetal nuclei are discharged into the microsporangium and form a plasmodium. Later the plasmodial substance is absorbed and the nuclei degenerate.

Gates and Rees[9] working on *Lactuca* have observed a four-nucleate condition of the tapetal cells of this plant. Cooper[5] mentions that in *Taraxacum. officinale* the mature tapetal cells are 8- to 16-nucleate. Recently Raghavan and Venkatasubban[18] have studied the behaviour of the tapetal cells in *Tridax procumbens*. It is interesting to note that their observations agree with my
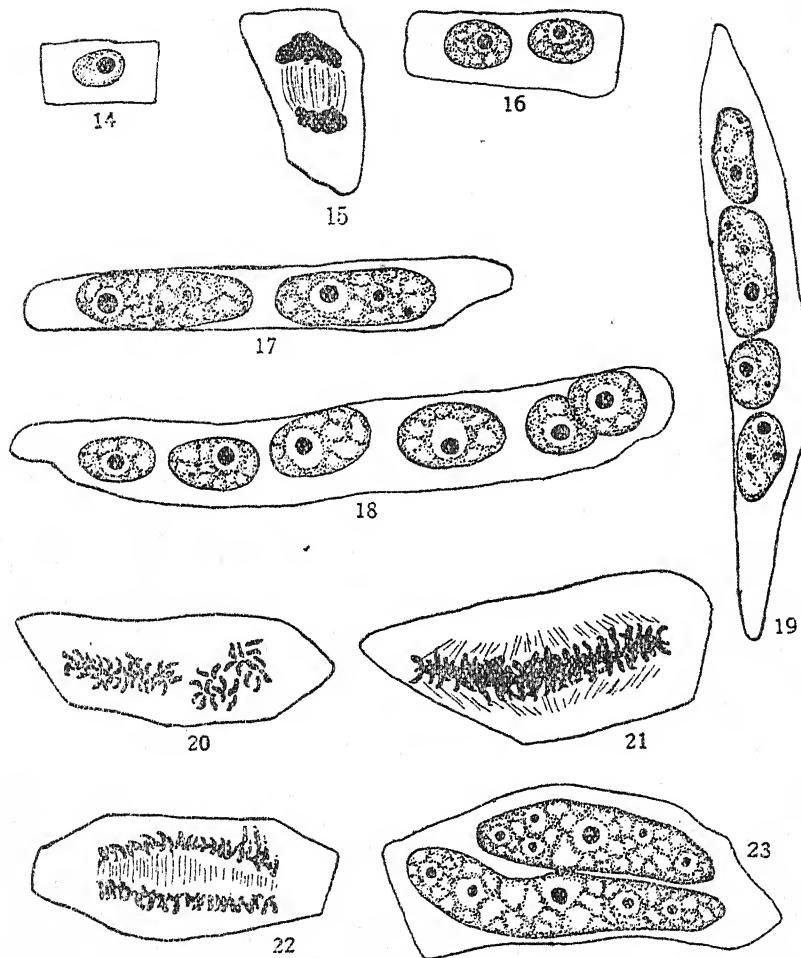
Text-figs. 14–23. *Blumea laciniata.*—Figures illustrate the nuclear condition of the tapetal cells. Explanation in text (×1167).

own on *Blumea laciniata,* except in one essential detail. They state that " more frequently the division of the tapetal nuclei does not result in the organisation of a definite daughter nucleus as such but there is a multiplication of chromosomes without any anaphasic separation. " Such a condition has, however, not been observed in the present material. Anaphasic separation of chromosomes has been observed in many preparations (Text-fig. 22), and the daughter nuclei appear to be well organised in every case (Text-fig. 23).

(4) *Megasporogenesis and development of the female gametophyte.*—The ovule arises as a tiny protuberance from the

base of the ovary when the microspore mother cells are first clearly recognised in the anther. A single hypodermal arches-porial cell is noted even before the curvature of the ovule is com-plete and this functions directly as the megaspore mother cell (Text-fig. 24). It passes through the usual stages of reduction division. The second division is illustrated in Text-fig. 25, after which a linear tetrad of megaspores is produced as usual. Datta[7] has stated that the megaspores become deeply buried inside the nu-cellar tissue as a result of the division of the cover cells. As pointed out by Venkateswarlu[23], this is not correct, for the megaspores always occur in a linear order below the epidermal layer of the nucellus. Incidentally, it might be mentioned that a similar condition prevails in *Launea asplenifolia* and *Mikania cordifolia*. Langlet[12], however, records a case in *Ambrosia maritima* in which several tapetal cells are cut off from the archesporial cell before it undergoes reduction division. The chalazal cell of the tetrad elongates in the direction of the micropyle and becomes functional. The remaining three megaspores degenerate (Text-fig. 26). The de-generated products remain as dark masses on the top of the functional megaspore. Disintegration of the nucellar epidermis is first noted at this stage. As the gametophyte reaches the two-nucleate stage it in-creases in size, as a result of which the surrounding nucellar cells are pushed apart and crushed. It now lies in direct contact with the integument, whose innermost cells elongate in a radial direction and form the so-called "integumental jacket". At the four-nucleate stage the nuclei become separated by cytoplasmic membranes (Text-fig. 27), so that in the eight-nucleate gametophyte the sister-nuclei relationship could be traced. In the mature gameto-phyte the synergids are pear-shaped structures with long beak-like processes which protrude into the micropylar cavity. They have conspicuous vacuoles at their bases, the nuclei being situated above. The egg projects below the synergids and has a large micropylar vacuole, the nucleus being embedded in the dense cytoplasm at the base. It extends as far as the synergids and is, as a rule, centrally situated. The polar nuclei fuse early and the secondary nucleus lies close to the egg. The three anti-podals are cut off as separate cells and lie mostly one above the other, but in a few preparations a different arrangement has been noted as is shown in Text-fig. 28. The mature gametophyte with all its parts fully organised is illustrated in the same figure.

(5) *The antipodal cells.*—The nuclei of the antipodal cells soon divide, but the division may or may not be followed by wall formation, the latter condition being more frequent. The maxi-mum number of antipodal cells seen in the present material was five, four being very common (Text-figs. 29, 39, etc.). Text-fig. 29 represents a group of antipodal cells which show a peculiar arrange-ment. The unusual orientation of the cells of this complex appears to be due to the division by oblique walls of the upper cell, after it had attained the binucleate condition.
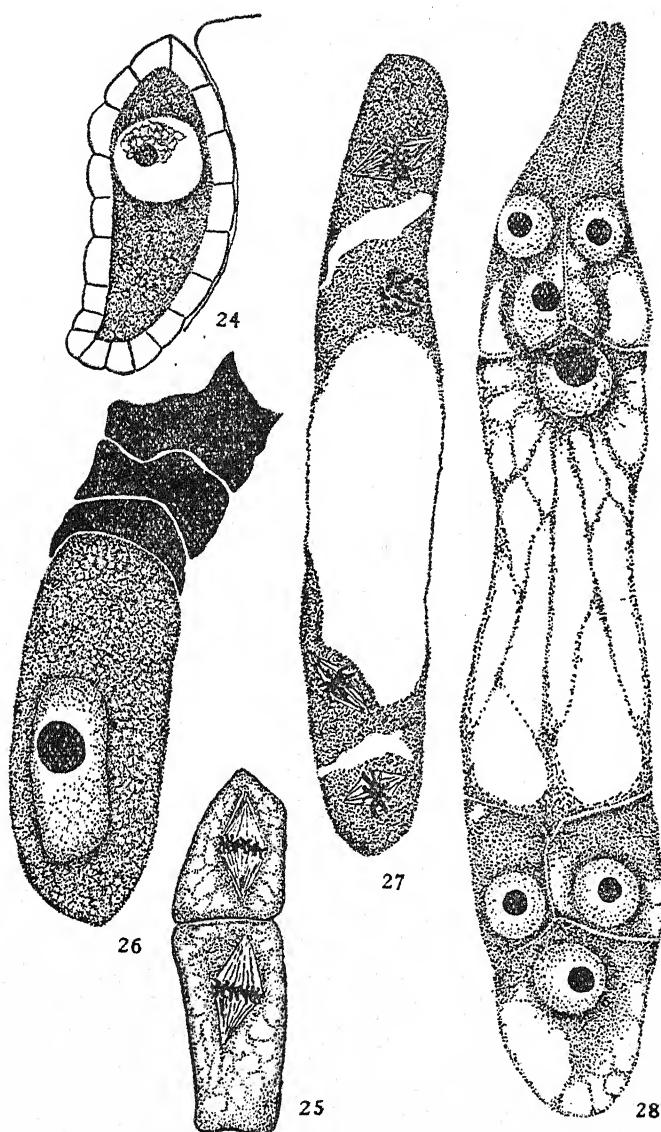
Text-figs. 24–28. *Blumea laciniata.*—Fig. 24. Megaspore mother cell in the hypodermal layer of the nucellus. Fig. 25. Homotypic division. Fig. 26. Functional and degenerating megaspores. Fig. 27. Quadri-nucleate embryo-sac. Fig. 28. Mature female gametophyte. Fig. 24 (×750); Fig. 25 (×340); Fig. 26 (×2200); Fig. 27 (×940); Fig. 28 (×1555).

In most of the antipodal cells two or more nuclei have been noted (Text-figs. 29–41). Where a single nucleus is present it is

generally bigger in size and shows the characteristic early pro-
phasic changes of mitosis. Uninucleate antipodal cells may thus
be considered to be of rare occurrence in this plant. In those cells
where more than four nuclei are present there is a tendency for
the nuclei to fuse (Text-figs. 37 and 41). This might be due to
the small space in which a number of nuclei are crowded together.
The maximum number of nuclei occurring in a single antipodal
cell has been found to be ten (Text-fig. 41). It will be seen that
the nuclei have partially fused and they appear as a conglomerate
mass. The antipodal cells normally degenerate during the dev-
elopment of the endosperm and appear as dark shapeless masses
which are later absorbed. Antipodal haustoria as recorded by
Howe[10] and others have not been observed.

An increase in the number of antipodal cells seems to be of
common occurrence in the family Compositæ. Small[20] states
that in *Senecio vulgaris* the basal or chalazal antipodal cell elongates
and produces as many as four antipodal cells each having one or
more nuclei. Reference to Text-figs. 29 and 30 will show that
increase in the number of antipodal cells in *Blumea laciniata* is
not due solely to the activity of the lowermost cell, the upper cell
may also take part in the process.

Development of an antipodal tissue consisting of a variable
number of cells has already been reported in the tribe Inuloideæ to
which *Blumea* belongs. According to Schnarf[19] *Antennaria* and
Gnaphalium show a variable number of antipodal cells. *Inula
helenium* has six and *Telekia speciosa* has three antipodal cells
each. Stebbins[21] found that the three antipodal nuclei of *An-
tennaria plantaginifolia* and *A. neglecta* divide repeatedly forming
cell membranes between them, so that at maturity there is an
antipodal tissue of 15 to 20 cells.

A multinucleate condition of the antipodal cells has also been
reported by many other workers. Bhargava[3] working on *Eclipta
erecta* observed that in certain instances the lower antipodal cell
has four, the middle two and the upper five nuclei. Täckholm[22]
reports the presence of as many as forty nuclei in a single anti-
podal cell of *Cosmidium burridgeanum*. *Bupthalmum salicifolium*
of the tribe Inuloideæ also shows the presence of multinucleate
cells.

(6) *Fertilisation.*—The embryo-sac increases in size before
fertilisation. The pollen tube enters the embryo-sac by way of
the micropyle and passes through one of the synergids which de-
generates. Porogamy appears to be the rule in this family. Stages
showing the actual entry of the pollen tube with the male nuclei
into the embryo-sac were not seen, but later stages in which the
male nuclei had been discharged into the embryo-sac cavity were
seen in many preparations. Text-fig. 42 shows a stage of fertili-
sation where the disorganised remnants of the pollen tube could
be seen at the micropyle with the tip lying close to the egg. In the
same figure the male nucleus is seen to lie adpressed to the nucleus
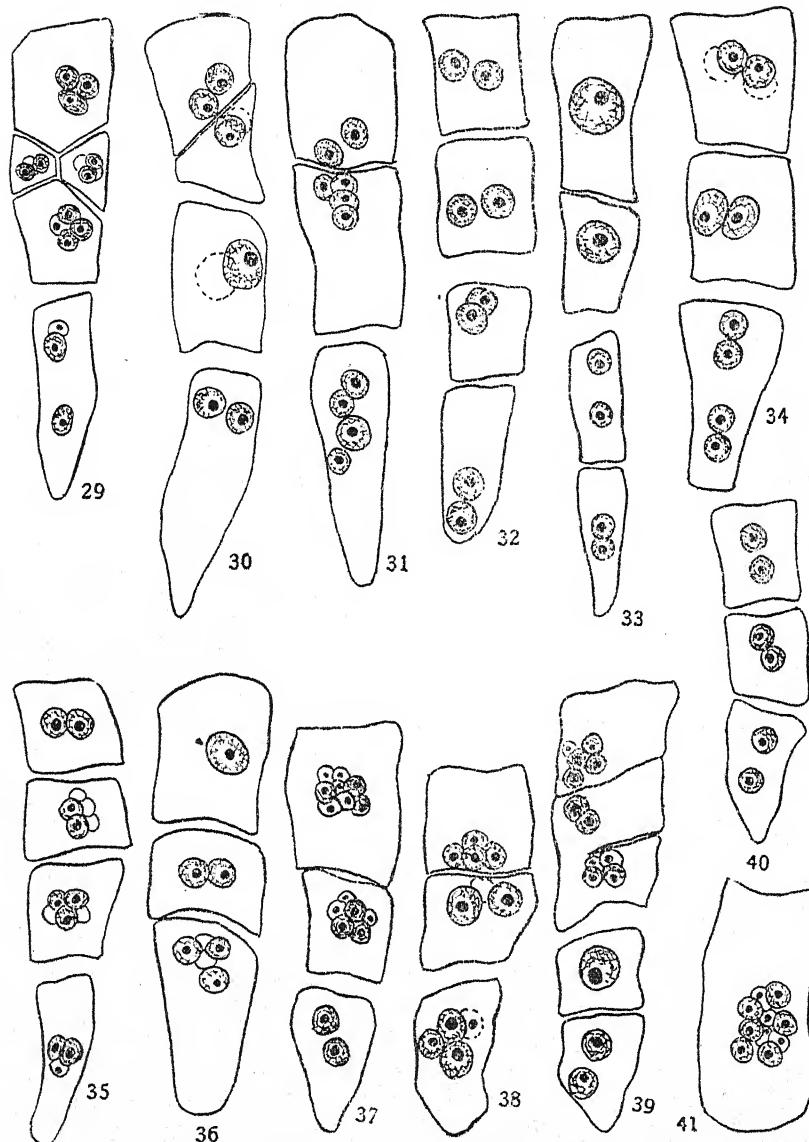
Text-figs. 29–41. *Blumea laciniata.*—Types of antipodals. Explanation in text (×1167).

of the egg and appears to be somewhat elongated. The second male gamete is also seen to lie against the nuclear membrane of the secondary nucleus. It thus appears that syngamy and triple fusion proceed more or less simultaneously. Two small dark bodies are also found to lie against the cytoplasm of the egg. Land[11]

found two similar structures inside the pollen tube in two species of *Erigeron* and in *Silphium laciniatum* and believes them to have come from a division of the tube nucleus. The presence of these structures near the egg in some preparations leads one to infer that they are the remnants of the degenerated tube nucleus.
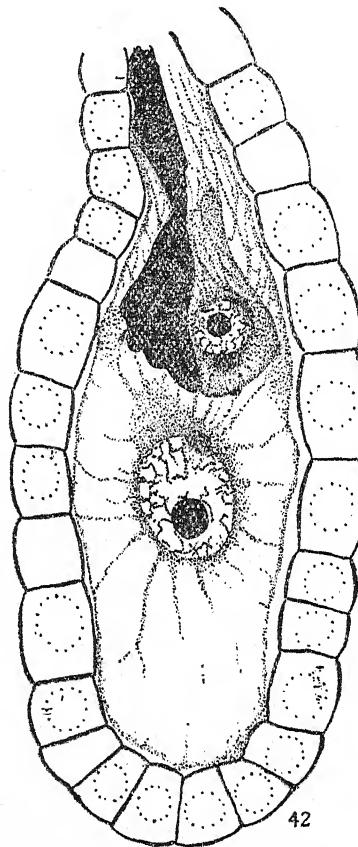
Text-fig. 42. *Blumea laciniata.*—Fertilisation (×900).

The form of the male nucleus as seen in *Blumea laciniata* is somewhat elongated. Nawaschin[15] observed a coiled appearance of the male nuclei in *Helianthus annuus* and Land[11] also reports the same in *Silphium laciniatum*. Howe[10] states that in *Grindelia squarrosa* " there seems to be no marked difference in size and shape between the egg nucleus and the male nucleus at the time of fertilisation ". Merrell[14] suggests that the male nuclei when first formed are spherical, but in the later stages they may be very much elongated and resolve into a spiral. Thus it appears that the form of the generative nucleus in the family Compositæ is variable at different stages.

(7) *The development of the endosperm and embryo.*— As is common in the angiosperms, the primary endosperm nucleus divides before the fertilised egg. The first few divisions are transverse and the cells become separated by distinct walls (Text-fig. 43), so that the endosperm is cellular from the beginning. Each cell contains
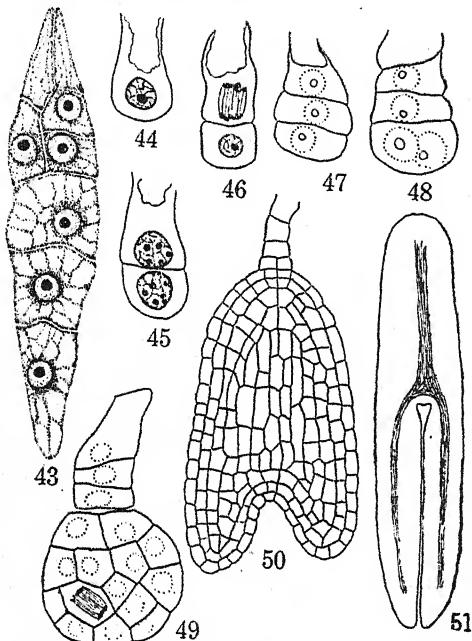
Text-figs. 43–51. *Blumea laciniata.*—Fig. 43. Cellular endosperm. Figs. 44–51. Stages in the development of the embryo. Fig. 43 (×30); Figs. 44–49 (×470); Fig. 50 (×225); Fig. 51 (×60).

a single nucleus which in the earlier stages of development shows the presence of two or more nucleoli. The later divisions of the cells take place in various planes and at an early stage of development the embryo becomes completely surrounded by the endosperm cells. A cellular endosperm appears to be characteristic of the tribe Inuloideæ, and it has been recorded in *Antennaria, Gnaphalium* and *Helichrysum* (see Schnarf, 1931).

The first division of the fertilised egg is transverse, resulting in the production of an upper basal cell and a lower terminal cell (Text-fig. 45). The basal cell soon divides by a periclinal wall and a three-celled pro-embryo is produced (Text-fig. 47). Division of the terminal cell soon follows and the lowermost cell soon undergoes longitudinal septation (Text-fig. 48). Stages immediately following this have not been observed. The next stage observed is represented in Text-fig. 49 where a globular embryo is seen with a three-celled suspensor. At a little later stage the cotyledonary lobes become differentiated and tissue differentiation in

the embryo appears to be complete (Text-fig. 50). The mature embryo fills up the seed completely, no endosperm being left. Between the two cotyledons the plumule is situated as a tiny protuberance and the plerome strands of the cotyledons emerge and meet the plerome of the root in the hypocotyledonary region. The cells of the plerome are radially elongated (Text-fig. 51). The suspensor is not found at this stage, probably it has been crushed by the rapidly developing sporophyte. The cells composing the embryo are full of reserve matter.

SUMMARY

The paper gives an account of the development of the florets, pollen-grains, tapetal cells, female-gametophyte, endosperm and embryo of *Blumea laciniata*. A short account of the process of fertilisation is also included.

1. The development of the floral organs takes place in the following sequence :—petals, sepals, stamens and carpels. In some flower-heads the central florets appear to develop earlier than the peripheral ones.

2. The pollen grains are formed by simultaneous division. The mature pollen grains are tri-nucleate. The exine shows the presence of spinous processes and furrows.

3. The tapetal cells of the anther first become binucleate and then multinucleate. The nuclei fuse and then divide, so that binucleate cells are produced again. The tapetal cells ultimately form a plasmodium.

4. The development of the female gametophyte is of the normal type. Three antipodal cells are seen in the mature embryo-sac. Some of these cells divide and four or five antipodal cells result, which generally become multinucleate, showing up to 10 nuclei.

5. Fertilisation is porogamous. The fusion of the male nuclei with the egg and polar nuclei appears to be simultaneous.

6. The endosperm is of the cellular type. Embryo development shows no unusual features.

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6*b*

A NEW PLANT FROM SOUTH BURMA

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While exploring the primeval rain forest in the hilly tracts of the unchartered area of Tenasserim (Mergui District, South Burma) along the border of Thailand, an interesting plant was discovered among the taller shrubby vegetation which forms a belt over the herbaceous community among the Bamboo and cane association. This zone is overtopped by the lofty Dipterocarpus-Dillenia-Sterculia association. The zigzag loops and arching veins of the leathery leaves coated with an epiphytic brown filamentous alga (?) attracted my notice. In 1930 I failed to obtain flowers but in a subsequent search in 1931 ample flowering materials were available. The plant cannot be matched with any species known to science but it bears a similarity to *Goniothalamus Meeboldii* of Craib, whose generic identification was hitherto doubtful. I have just been able to establish its correct identity as sufficient duplicate flowering materials are now available. Both *G. Meeboldii* and this new plant, which I name as *Goniothalamus tenasserimensis* Biswas, are recorded from Lower Burma. The Tenasserium specimen however is entirely different from the Andaman specimens of *G. Meeboldii* gathered by Parkinson and King's collector. Nevertheless it resembles to a certain extent the fruiting type specimens of *G. (?) Meeboldii* No. 17250. A description of the new species together with sketches of my type sheet is appended.

Goniothalamus tenasserimensis Biswas, Species *Nova, affinis G. Meeboldii* Craib, sed foliis oblongo-lanceolatis; nitidis, nervis obliquis, parallelis distantis, supra haud impressis, infra prominentibus margine valde arcuato-anastomosantibus; pedunculi uniflori, frequenter supra-axillares; flores leviter parvis, stylus non divisus, late curvatus differt.

Tenasserim, Kyenchong Forest (border of South Burma) Thailand, 70 m, K. Biswas, No. 1195. (Typus in Herb. Calcutt.; duplum. No. 1225 in Herb. Calcutt.)

DESCRIPTION

A small tree 3-5 mm. tall, young branches hairy or tomentose, hairs brown; bark dark-brown, minutely fissured. *Leaves* somewhat cunneate, oblong lanceolate or narrowly elliptic, acute or more or less acuminate, chartaceous or subcoriacious, margin entire, more or less cartilaginous, slightly revolute; lamina 10-22 cm. long 2·9 to 5·5 cm. broad; upper surface glabrous, somewhat smooth,

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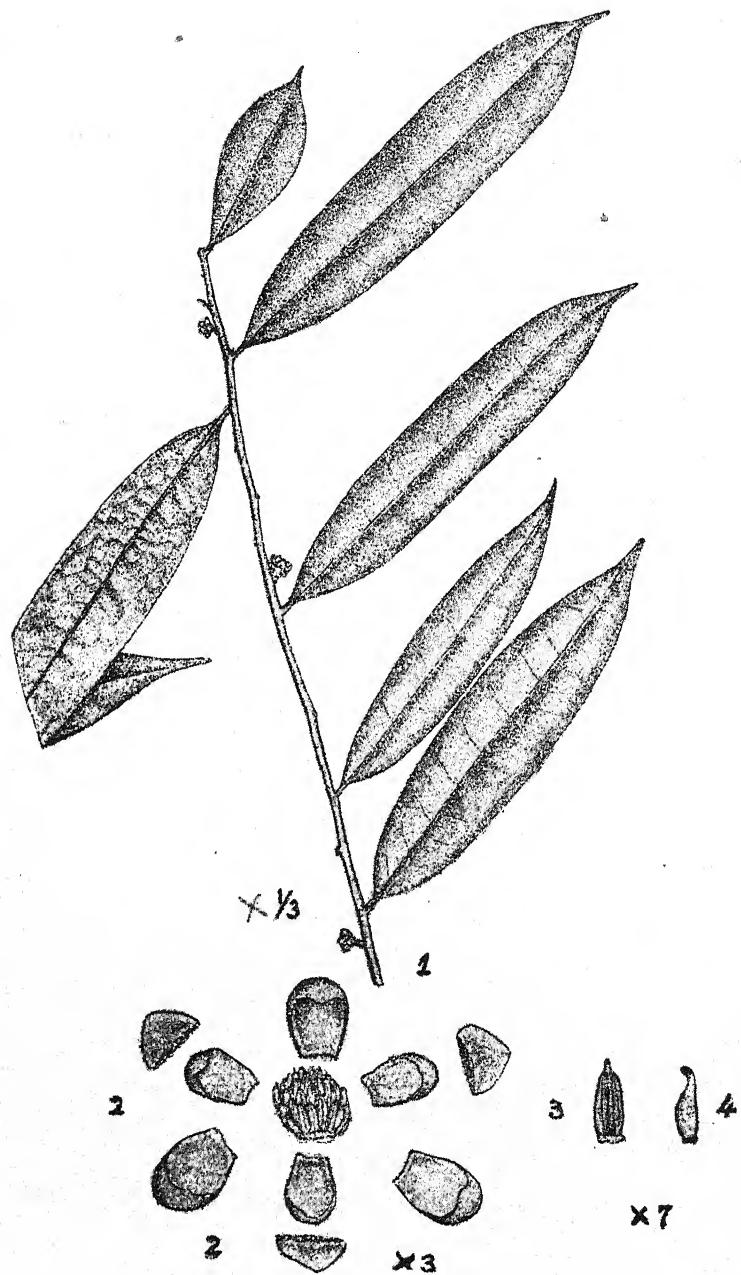
Fig. 1. A portion of the branch with flowers.   × 1/3.   Fig. 2. A flower dissected.   × 3.   Fig. 3.   Stamen.   × 7.   Fig. 4.   Ovary with style and stigma, × 7.

dirty green, lower smooth, glaucous, lateral nerves 8-14 pairs, arching over and anastomosing from the margin towards the midrib, distance between the arches 2-5 mm., midrib distinctly channelled throughout on the upper surface; petiole stout 4 mm. long. *Flowers* solitary, almost always extra or supra-axillary, shortly pedicelled, glandular; sepals 3, valvate smaller than the petals, deltoid, ovate, 2·5 mm. long, 3 mm. broad at the base; petals 6·7 mm. long 3·5 mm. broad free, subequal in two rows, thick hard ovoid, incurved, all the petals opening simultaneously in flowers, pubescent and glandular on the outside. Stamens many, sessile; anthers lateral, connective 1·5 mm. long, flat, truncate. Carpels many (more than 10), elliptic; style very short, about 1 mm. long. Stigma capitate; ovule solitary, basal; torus convex. Fructus non-visa.

Habitat.—Kyenchong Rain Forest, Tenasserim (border of Thailand and South Burma), Mergui District on low hills about 200 ft. in elevation—Mostly along the riverside of the Kyenchong river. Type No. 1195, duplicate No. 1225, collector—K. Biswas.
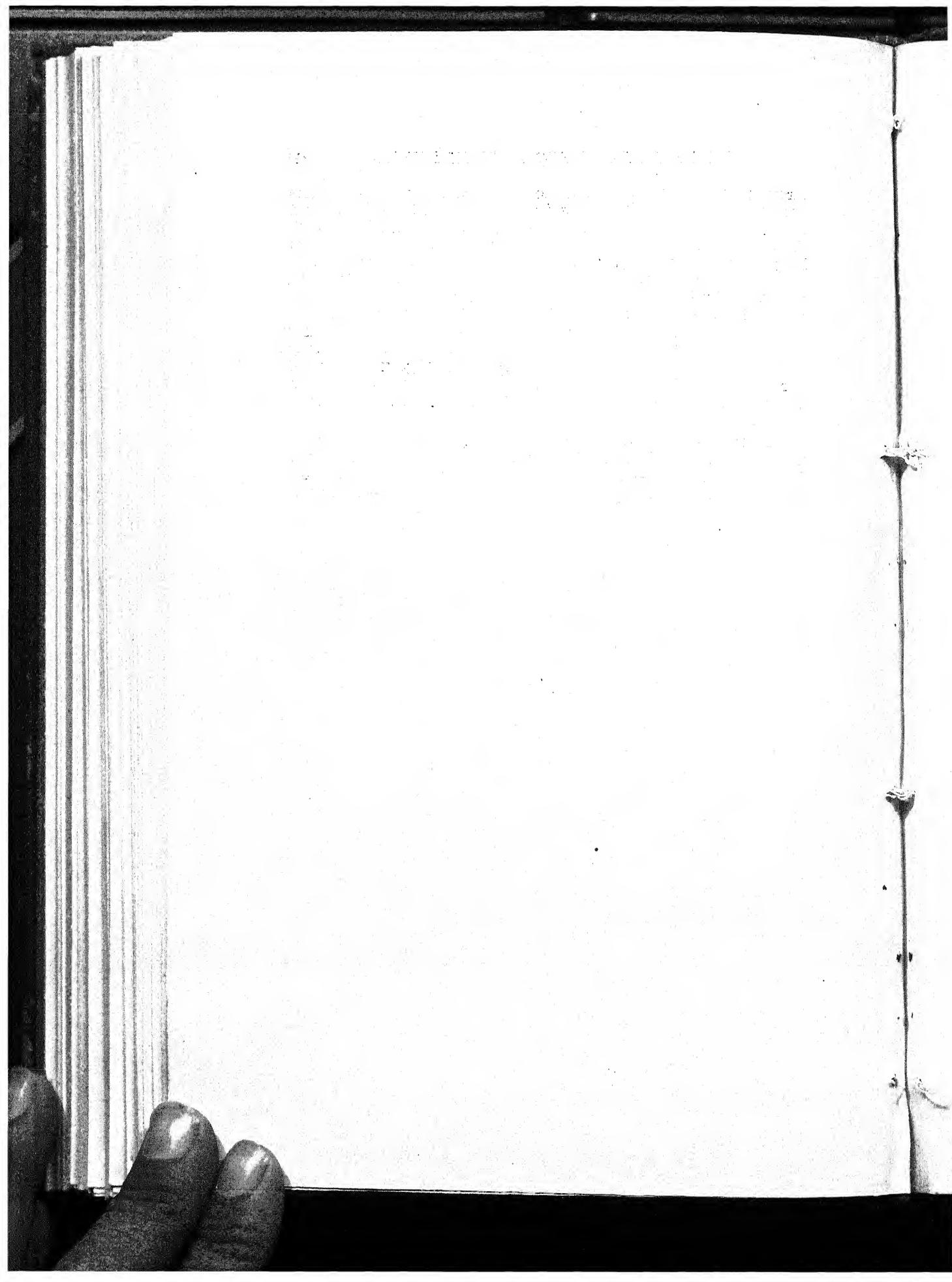
THE GENUS, *CHELONOPSIS* MIQ., RECORDED FOR THE FIRST TIME FROM INDIA

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(Communicated by Dr. K. Biswas)

Chelonopsis Miq. is a Sino-Japanese genus belonging to the family—Labiatæ. The species *C. albiflora* Pax and Hoff., was collected from Batang situated on the border-line between China and Tibet. This was the western limit of the genus known hitherto. Recently P. N. Kohli collected a new *Chelonopsis* from the Uri hills in Kashmir. This discovery brings the genus within the scope of the Indian flora, and the range of distribution of this genus is thus extended to a distance of about 1,000 miles further west.

The genus *Chelonopsis* since it was described in 1865 was considered to be monotypic. In 1890 Hemsley combined *Bostrychanthera* Bth. with *Chelonopsis* Miq., transferring *Bostrychanthera deflexa* Bth. et Hk. f. to *Chelonopsis* and named this species as *Chelonopsis Benthamiana* Hemsl. Briquet in Engler und Prantl's *Naturliche Pflantzenfamilien* followed Bentham and placed *Bostrychanthera* under Prasioideæ and *Chelonopsis* under Stachydeæ. Recent authors, *viz.*, Druce, Diels, Dunn and Kudo, have accepted the views of Hemsley and treated *Bostrychanthera* as synonymous to *Chelonopsis*. The conclusions arrived at by Bentham and also by Briquet are based upon immature fruits. Detailed examination of mature fruits however by different authors definitely established that *Bostrychanthera* should not be treated as distinct from *Chelonopsis*.

The plant collected from Kashmir by Kohli is allied to *C. albiflora* Pax and Hoff., and also to *C. Forrestii* Antony. This plant is treated here as a new variety of *C. albiflora*. A short description of the genus *Chelonopsis* together with a key to all the species known up to the present time is given in this paper.

Chelonopsis Miq.

In Ann. Mus. Bot. Ludg., Bat. II, p. 111 (1865), et Prodr. Fl. Jap., p. 43 (1866); Benth. and Hk.f. Gen. Pl. II, p. (1873); Maxim. in Mel. Biol., IX, p. 443 (1873); Briq. in Engl. und Pr. Nat. Pflanzenf. IV, 3, a, p. 243 (1897) ; Kudo in Mem. Fac. Sc. Agri., Taihoku II, 2, p. 151 (1929).

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Bostrychanthera Benth. in Benth. & Hk.f. Gen. Pl. II, p. 1216 (1873) ; Briq. in Engl. und Pr. Nat. Pflanzenf., IV, 3, a, p. 223 (1897).

Herbs or *shrubs ; leaves* widely toothed. *Flowers* large in lax axillary cymes, or flowers often solitary. *Calyx* membranous, campanulate or bilabiate, 10-nerved, upper lip 3-toothed, lower 2-toothed. *Corolla-tube* somewhat expanded from near the base, slightly bent in front, exannulate, limb 2-lipped, upper lip emarignate, lower 3-lobed, the median the largest. *Stamens* 4, didynamous, inferior longer, filaments compressed, anthers 2-locular, cells distinct, divericate, penicellate. *Disc* equal. *Style* subequally 2-fid, lobes subulate. *Nutlets* compressed on the dorsal side, apex produced to an oblique wing.

A. Leaves distinctly petioled,
   B. Leaf margin not ciliate,
      C. Leaves membranous,
         D. Bracts inconspicuous, not foliaceous,
            E. Leaves lanceolate, acuminate,
               6 cm. long or more,
                  F. Flowers purple, teeth of
                    lower calyx-lip much longer,   .. moschata
                  F. Flowers white or pale yellow,
                    calyx-teeth subequal,   .. albiflora
            E. Leaves ovate, acute,
               3 cm. long,   .. Giraldii
         D. Bracts conspicuous, foliaceous,
            E. Leaf-blade 8 cm. long,
               corolla yellow,   .. Smithii
            E. Leaf-blade 10–15 cm. long,
               corolla deep rose,   .. bracteata
      C. Leaves chartaceous,
         D. Flowers yellow,
            E. Peduncle 5 cm. long,   .. lichiangensis
            E. Peduncle 1-2 cm. long,   .. odontochila
         D. Flowers deep rose,   .. rosea
   B. Leaf-margin ciliate,
    · C. Leaf-apex caudate-acuminate,   .. Yagiharana
     C. Leaf-apex long acuminate,
        D. Flowers white,   .. Forrestii
        D. Flowers purple-rose   .. siccanea
A. Leaves sessile   .. deflexa

1. Chelonopsis moschata Miq. in Ann. Mus. Bot. Ludg. Bat. II, p. 111 (1865), et. Prod. Fl. Jap. p. 43 (1866) ; Maxim. in Mel. Biol. IX, p. 443 (1873); Fr. et Sav. Enum. Pl. Jap. I, p. 378 (1875) ; Forb. et Hemsl. Ind. Fl. Sin. II, p. 298 (1890) ; Hk. f. in Curtis's Bot. Mag. 127, pl. 7783 (1901) ; Briq. in Eng. und. Pr. Nat. Pflanzenf. IV, 3, a, p. 243 (1897) ; Matsum. Ind. Pl. Jap. II, p. 537 (1912) ; Matsum *et* Kudo in Tokyo Bot. Mag., XXVI, p. 297 (1912) ; Kudo in Mem. Fac. Sc. Agri. Taihoku II, 2, p. 151 (1929).
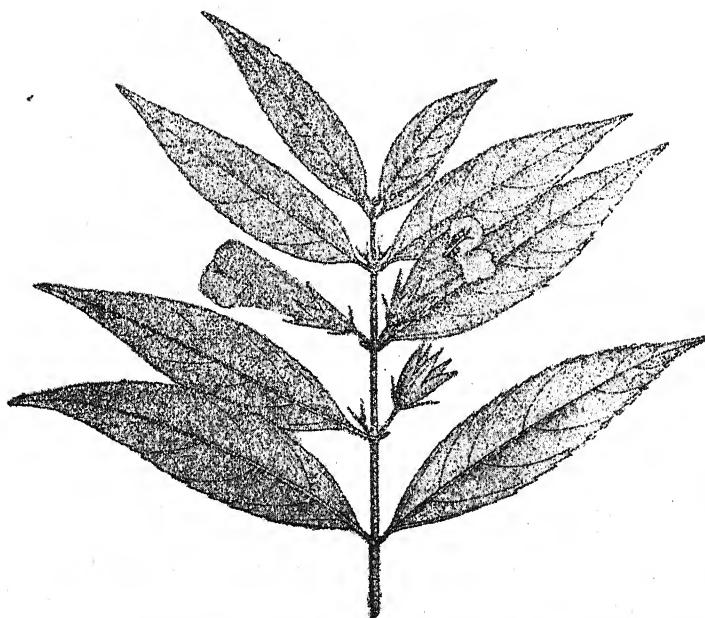
Chelonopsis albiflora Pax. & Hoff. var. *cashmirica* Mukerjee var. nov.
(½ Nat. size.)

Dist. Japan; China–Ningpo Mts.

Var.—**longipes** Makino in Tokyo Bot. Mag. VI, p. 54 (1892);
Kudo in Mem. Fac. Sc. Agri. Taihoku II, 2, p. 152 (1929).

Chelonopsis longipes Makino in Tokyo Bot. Mag. XII, p. 57
(1898); Mastum Ind. Pl. Jap. II, 2, p. 537 (1912).

Dist. Japan–Honshu.

2. *Chelonopsis albiflora* Pax *et* Hoffman in Fedde Rep. Beih.,
XII, p. 477 (1922).

Dist. E. Tibet,–Batang.

Var.—**cashmerica** Mukerjee, var. nov.

Haec varietas a typo foliis majoribus, florum pedunculis
longioribus, corolla flava longiore, recedit.*

Small *shrub*, with slender, puberulous stem. *Leaves* shortly
petioled, membranous, lancelolate, acuminiate, acute at the base,
margin sharply and distantly serrate, entire near the base, upper-
side glandular and sparsely hairy with minute scattered hairs,
lower side glandular-punctate, densely hairy on the nerves with
white somewhat floccose hairs; lamina 8–10·5 cm. long, 1·5–3 cm.

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* I am indebted to Dr. N. L. Bor for the Latin portion of the
description. S. K. M.

broad ; petiole 4–6 mm. long, densely floccosely hairy with white and glistening hairs ; nerves 6-7 pairs, prominent beneath. *Cymes* one-flowered in the axil of leaves near the end of the branches ; bracts opposite, linear, 6-8 mm. long ; peduncle slender, pubescent with minute white hairs, 4 mm. long, elongated in fruit ; pedicels 1–2 mm. long. Calyx campanulate, 15 mm. long, deeply 5-toothed, 10-nerved, pubescent on the nerves with white glistening hairs ; teeth acuminate, ciliate at the margin, upper teeth 7 mm. long, lower 9 mm. long; fruiting calyx coriaceous, 20 mm. long. *Corolla* pale yellow, pubescent outside, with white floccose hairs, 3·5 cm. long ; upper lip 6 mm. long, slightly emarginate, lower lip 3-lobed, 11 mm. long, 25 mm. broad, the mid-lobe much the largest. *Fillaments* pubescent, anthers penicellate. *Nutlets* obovate, compressed, winged on top, 3 mm. long.

Dist. Uri Hills Kashmir, 2,000 Mtr., P. N. Kohli No. 189 (type in Herb. Cal.).

This sheet was sent to the writer by Rev. Dr. R. R. Stewart, Principal,—Gordon College, Rawalpindi, who kindly permitted me to describe it. There are two other sheets of this number at the Gordon College Herbarium.

3. **Chelonopsis Giraldii** Diels in Eng. Bot. Jarb. XXXVI, Beibl. Nr. 82, p. 94 (1905) ; Dunn in Notes R.B.G. Edin. No. XXVIII, p. 177 (1915) ; Kudo in Mem. Fac. Sc. Agri. Taihoku II, 2, p. 153 (1929).

Dist. China,—Schensi.

4. **Chelonopsis Smithii** (Kudo) Mukerjee Comb. Nov.

Chelonopsis odontochila Diels. subsp. *Smithii* Kudo in Mem. Fac. Sc., Agri. Taihoku II, 2, p. 154 (1929).

Dist. China—Yunnan.

5. **Chelonopsis bracteata** W. W. Smith in Notes, R. B. G. Edin. No. XLII, p. 92 (1916).

Chelonopsis odontochila Diels, subsp. *bracteata* Kudo in Mem. Fac. Agri. Sc. Agri. Taihoku II, 2, p. 154 (1929).

Dist. China—Yunnan.

6. **Chelonopsis lichiangensis** W. W. Smith in Notes R. B. G. Edin. No. XLII, p. 92 (1916).

Chelonopsis odontochila Diels. subsp. *Lichiangensis* Kudo in Mem. Fac. Sc., Agri. Taihoku II, 2, p. 154 (1929).

Dist. China—Yunnan.

7. **Chelonopsis odontochila** Diels in Notes, R. B. G. Edin. No. XXV, p. 240 (1912) ; Dunn in Notes R. B. G. Edin. No. XXVIII, p. 178 (1915).

Chelonopsis odontochila Diels. subsp. *odontochila* Kudo in Mem. Fac. Sc. Agri. Taihoku II, 2, p. 154 (1929).

Dist. China—Yunnan.

8. **Chelonopsis rosea** W. W. Smith in Notes, R. B. G. Edin. No. XLII, p. 93 (1916).

Chelonopsis odontochila Diels. subsp. *rosea* Kudo in **Mem.** Fac. Sc. Agri. Taihoku II, 2, p. 155 (1929).

Dist. China—Yunnan.

9. **Chelonopsis Yagiharana** Hisauchi *et* Matsum in **Journ.** Jap. Bot. II, P. 1, f. 1-2 (1918); Kudo in Mem. Fac. Sc. Agri. Taihoku II, 2, p. 152 (1929).

Chelonopsis moschata Miq. var. *lasiocalyx* Hayata in **Tokyo** Bot. Mag., XXXII, p. 252, *et* Icon. Pl. Form. VII, p. 110 (1918).

Dist. Japan—Honshu.

10. **Chelonopsis Forrestii** Anth. in *Sched.* **Herb.** R. B. G. Edin.; Notes R. B. G. Edin. XV, p. 239 (1927).

Chelonopsis odonotochila Diels. subsp. *Forrestii* Kudo in Mem. Fac. Sc. Agri. Taihoku II, p. 154 (1929).

Dist. China—Szetschwan.

11. **Chelonopsis siccania** W. W. Smith in Notes, R. B. G. Edin. No. XLII, p. 94 (1916).

Chelonopsis odontochila Diels. subsp. *siccanea* Kudo in Mem. Fac. Sc., Agri. Taihoku II, 2, p. 155 (1929).

Dist. China—Yunnan.

12. **Chelonopsis deflexa** Bth. & Hkf. Diels. Fl. C. China P. 554; Dunn. in *Notes* R. B. G. Edin. No XXVIII, p. 178 (1915); Kudo in Mem. Fac. Sc. Agri. Taihoku II, 2, p. 152, 1929.

Bostrychanthera deflexa Bth. & Hkf. Gen. Pl. 2216 ; Briq. in Eng. und. Pr. Nat. Pflanzenf. IV, 3, a, p. 223 (1897).

Chelonopsis Benthamiana Hemsl. In Forb. *et.* Hemsl Ind. Fl. Sin. II, p. 298 (1890).

Dist. China,—Hupeh, Fukien.
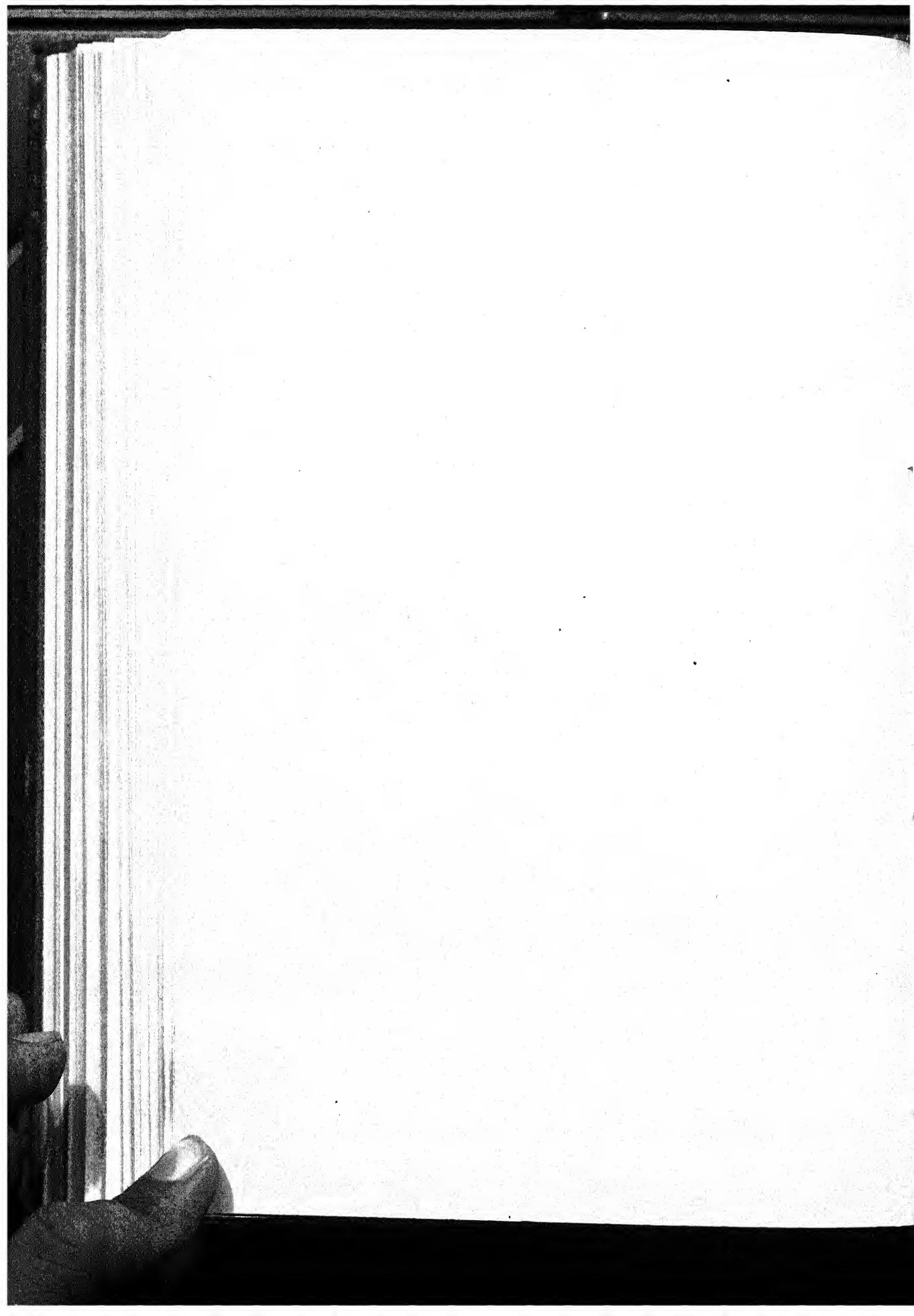
THE ANATOMY OF THE STEM, LEAF AND PETIOLE OF *ZANONIA INDICA, L.*

By Balwant Singh

Department of Biology, Dacca University

Introduction

Zanonia indica is a climber belonging to the family Cucurbitaceæ, reported to occur in India, Ceylon and Malaya. In 1938 a flowering specimen was collected by Mr. S. K. Sen from a mango tree growing by the side of the Dacca-Manipur road ; in January, 1941, the plant was found to have been severed from its roots, but although leafless, it was not dead and was producing long ærial roots, as in *Tinospora cordifolia*. Cuttings of the stem struck root when planted and duly developed into new plants.

The plant has since been also found at several other places in the district of Dacca. A large leafless but living specimen, of which the terminal portion was missing, measured 54 ft. from its cut basal end, which was 14 ft. above the ground and was sending down long ærial roots some of which had already reached the soil.[1]

A casual examination of the stem failed to show any intraxylary phloem. Since such a condition is rare in Cucurbitaceæ, I was led to make a detailed investigation of the plant. The present paper deals with the anatomy of the stem, leaf and petiole. The study of the roots, both terrestrial and ærial, will be treated in a subsequent paper.

Previous Work

Haberlandt (1914), De Bary (1884) and Engler-Prantl (1897–1915) have all stated in a general way that bicollateral bundles are characteristic of the family Cucurbitaceæ. Petersen[2] (1882) stated that while the outer phloem always forms an integral part of the bundle, this is not to the same degree the case with the inner phloem. Thus, in the stem of *Alsomitra sarcophylla*, he (1882) found that the intraxylary phloem was replaced by "cambiform tissue".[3] Similarly Fischer (1884) failed to find internal phloem in one species of *Gerrardanthus* and Schenck (1893) in *Anisosperma Passiflora*.[4]

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[1] A detailed note on the habit and habitat of *Zanonia* by Mr. S. K. Sen is in the press.

[2] Quoted in Worsdell (1915).

[3] This was confirmed by Fischer (1883, 1884) and Hérail (1885) (see Solereder, 1908, p. 394 for references).

[4] See Solereder (1908, p. 397) for references.

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Wallace[5] working on the stem structure of *Actinostemma biglandulosa*, found that even after considerable secondary growth all the bundles are collateral. Medullary phloem appears at a much later stage but it does not arise simultaneously in all the bundles.

Solereder (1908) writes that probably *Feuillea cordifolia* and *Gynostemma integrifolia* also lack intraxylary phloem and in *Zanonia indica* " bicollateral structure does not, at any rate, extend to all the bundles ". Zimmerman (1922) says that among the plants that he investigated, two (*Gerrardanthus grandiflorus* and *Cyclantheropsis parviflora*) had collateral bundles.

MATERIAL AND METHODS

The material was fixed in formalin-acetic-alcohol and sectioned at 12–20 μ. The older stems were treated with hydrofluoric acid before embedding. The sections were stained with Safranin and Fast Green. Macerations were also made according to the method given by Jeffrey (1917).

ANATOMY OF THE YOUNG STEM

The stem is at first five-angled in cross section (Fig. 1) but afterwards becomes circular due to secondary changes. The epidermis consists of a single layer of cells. Stomata occur usually in the furrows, and lie in the same line as the other epidermal cells. The epidermal hairs are of two different types, long uniseriate (Fig. 2) and short glandular (Fig. 3).

Next to the epidermis is the collenchyma (Fig. 4). This is especially well developed at the angles where it consists of 3–4 layers of large polygonal cells, whereas in the furrows it consists of only one to two layers of slightly smaller cells. The rest of the cortex consists of chlorenchymatous cells forming 2–3 layers at the ridges and 3–5 at the furrows (Fig. 4).

The endodermis could not be made out clearly. Towards the inside of the cortex there is a continuous wavy band belonging to the pericycle and consisting of 4–8 layers of sclerenchymatous cells (shown cross-hatched in Fig. 1) forming broad arches over the vascular bundles.[6] The cells of the outer layers are much smaller and more lignified as compared to those on the inside.

On the inner side of the sclerenchymatous band there are 1–2 layers of thin-walled parenchyma, forming the " extrafascicular ground tissue " of Zimmerman (1922). These cells have no chloroplasts.

Typically there are ten vascular bundles arranged in two rings. The outer bundles are situated beneath the ridges while the inner

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[5] Quoted in Worsdell (1915).

[6] Various names have been given to this sclerenchymatous band. Kundu (1942) proposes to call it " peristelar sclerenchyma ".
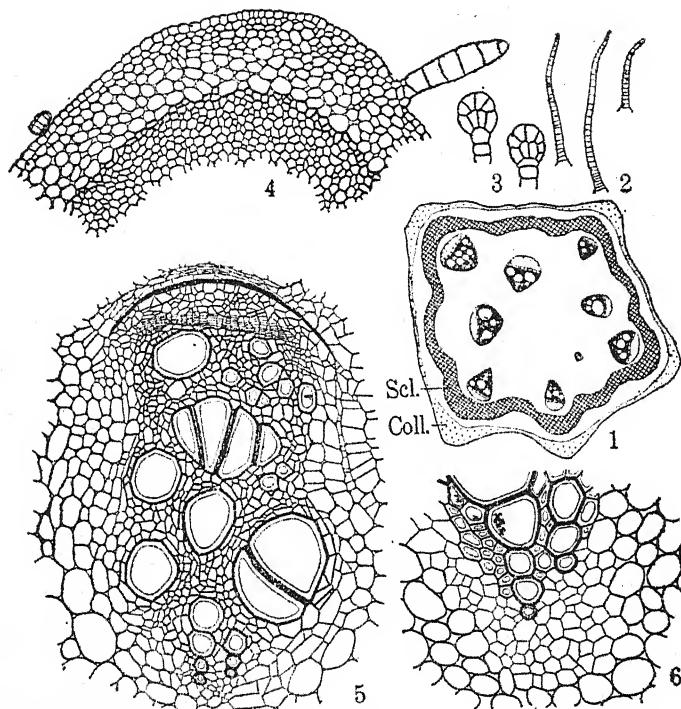
Figs. 1–6.—Fig. 1. Diagram of t.s. of a young stem (×22). Figs. 2, 3. Uniseriate and glandular hairs from scrapings of a young stem (×22 and ×125 respectively). Fig. 4. Part of a transverse section of a young stem showing the epidermis, collenchyma, cortex and thick-walled pericycle (×105). Fig. 5. A single vascular bundle in t.s. Note absence of internal phloem (×70). Fig. 6. Inner portion of an older bundle showing the small-celled parenchyma or " cambiform tissue " towards the inside of the protoxylem (×140).

alternate with them and lie below the furrows. In the young stem the outer ring has 5 bundles while the inner has often only 4, the fifth one appearing later (Fig. 1). Occasionally there are seven bundles in the outer ring (with the normal 5 in the inner) and corresponding to these the stem also shows seven angles. In such cases four of the bundles are smaller than the remaining three and are seen in pairs.

All the bundles lack internal phloem, a feature which is rare in the Cucurbitaceæ (Fig. 5).

Tyloses are occasionally present in the xylem vessels. Just inside the protoxylem there are a few small thin-walled parenchymatous cells occupying the position of the intraxylary phloem (Fig. 6). They were never found to have any sieve tubes, however, and are evidently similar to the " cambiform tissue " mentioned by Petersen in *Alcomitra*.

Anatomy of the Stem after Secondary Growth

Solereder (1908, p. 396) says that cork formation is rare and takes place only in relatively thick stems of *Zanonia*. This is not borne out by the present investigation and it appears that he did not have sufficient material for his observations.[7] In my material cork formation was found to have begun even in young stems. In most of the Cucurbitaceous plants the cork cambium is said to arise in the first or second layer below the epidermis (Zimmerman, 1922). In *Zanonia* it usually has a slightly deeper origin arising underneath the collenchyma in the angles and in the second layer at other places.[8] Only in a few pieces collected during the rains the cork cambium was found to have originated in the second layer of collenchyma at the angles (Fig. 8), and just below the epidermal layer at other places (Fig. 9).

In slightly older stems the epidermis is almost completely replaced by cork except at the angles where it persists a little longer (Fig. 7). Lenticles usually occur opposite to the medullary rays. In the oldest stems the cork consists of several alternating layers of thin and thick-walled cells. The latter are greatly compressed and appear as dark wavy bands under a lens (Fig. 10). As the stem increases in size, the older layers of cork are sloughed away and their place is taken by younger cork formed by successive cylinders of phellogen which are produced centripetally. A curious feature of the periderm is that some portions of the sclerenchymatous band become included in it. This will be considered later in connection with the pericycle.

Towards the inner side, the phellogen cuts off 6–10 layers of phelloderm. The younger cells are rectangular in shape and radially arranged, while the older ones are elliptical with intercellular spaces in between them. They are rich in starch grains. The primary cortex becomes greatly reduced and narrowed. A few stone cells also appear inside and opposite to the medullary rays (Fig. 10).

The wavy band of pericycle, which was continuous in the young stem (Figs. 1 and 11a) begins to break up into crescent-shaped bands (Fig. 11 b) overarching the vascular bundles. Some of the outermost cells of this band become still further sclerised and form stone cells. A few stone cells also arise between some of the pericycle arches forming a sort of bridge from one band to another. In some of the stems an additional phellogen was found to arise below some of the arches (Fig. 11 c). Due to its activity the latter are gradually pushed outwards and become surrounded on all sides by a cork cambium (Fig. 11 d). As the growth is more vigorous below the patch, it is eventually pushed out through the first cork cambium into the old cork (Fig. 11 e).

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[7] It is probable that his observations were based on herbarium mounts of very thin twigs only.

[8] *Cf. Momordica trifoliata* and *M. rostrata* (Zimmerman, 1922).
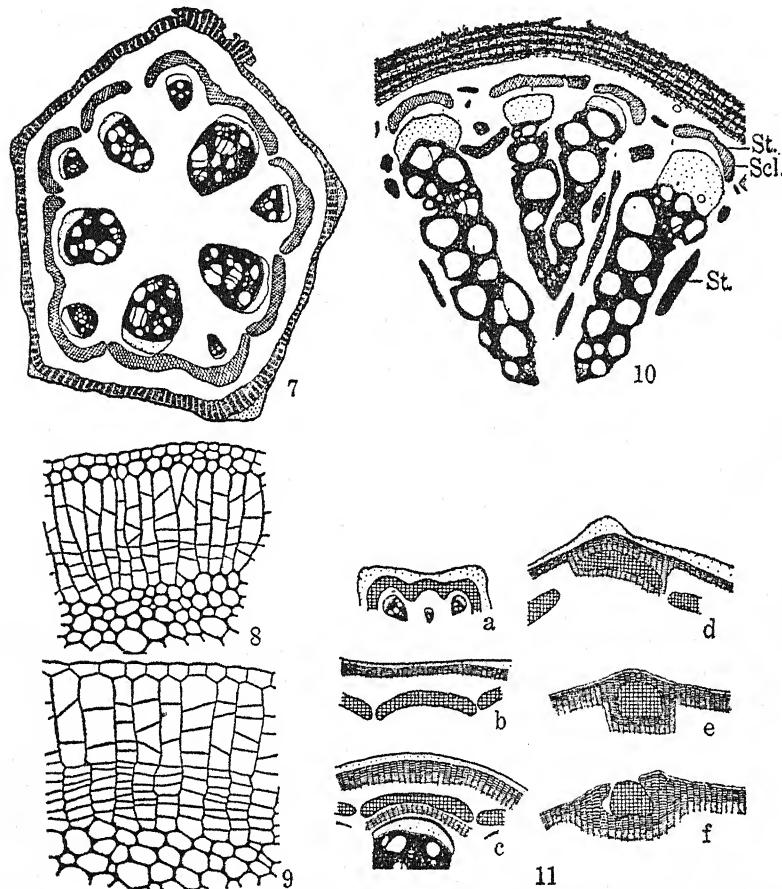
Figs. 7–11.—Fig. 7. Diagram of t.s. of older stem showing beginnings of cork formation. The collenchyma is persisting at the angles. The sclerenchymatous ring has started breaking into separate arches over the vascular bundles (×16). Fig. 8. Portion of t.s. of a twig in the angular portion showing the development of cork in the second layer of collenchyma (×140). Fig. 9. Same, in the region of a furrow, showing the origin of cork from the layer just below the epidermis (×140). Fig. 10. Diagram of a portion of t.s. of a still older stem showing multilayered cork and radial elongation and splitting of vascular bundles (×13). Fig. 11 (a–f). Diagrams of portions of t.s. of stems showing different stages in the thrusting out and elimination of a portion of the sclerenchymatous pericycle (×21).

As mentioned before the vascular bundles are originally in two rings but as secondary growth advances the cambia of the inner and outer bundles become connected together so that the bundles now lie in a single ring. Some of the xylem elements become so large that they can be made out even with the naked eye. With the growth of the secondary phloem, the primary phloem is crushed to form a crescent-shaped mass.

As the stem increases in diameter the bundles (particularly the five larger ones) become radially split due to the formation of secondary vascular rays and the xylem consequently develops not as a solid mass but in the form of separate strands which are thin and wedge-shaped in cross section. This is a well-known phenomenon met with in all climbing plants and the breaking up of the originally continuous sclerenchymatous ring into small crescents is also to be interpreted similarly.

The interfascicular cambium continues to add new cells to the medullary rays. These cells are radially elongated and elliptical and like the pith they store large quantities of starch grains. At some places inside the rays there are small patches or streaks of stone cells which contain very small crystals, probably of calcium oxalate.

The pith becomes greatly reduced in size due to the radial elongation of the vascular bundles. Its cells get compressed and some are entirely obliterated. In certain sections stone cells have also been seen in the pith.

PETIOLE

The petiole possesses epidermal hairs similar to those on the stem (Fig. 12). The cortex is differentiated into collenchyma and chlorenchyma. There is no well-marked endodermis. The vascular bundles, usually 7 in number, are arranged in the form of a horse-shoe, the largest being situated opposite to the adaxial groove. In the younger stages there may be only 5 or 6 bundles. The structure of the vascular bundles is essentially similar to that in the stem except that in the petiole each bundle has a narrow cap of tangentially elongated parenchymatous cells instead of the sclerenchyma band found in the stem. The pith consists of large spherical cells with starch grains.
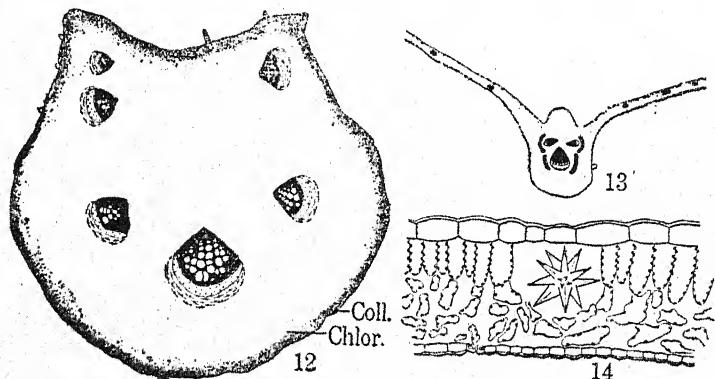
Figs. 12–14.—Fig. 12. Diagram of t.s. of petiole ($\times 26$). Fig. 13. Diagram of t.s. of leaf through the midrib ($\times 9$). Fig. 14. T.s. of leaf lamina ($\times 121$).

LEAF

Hairs of both uniseriate and glandular type are present. The palisade tissue consists of 1–2 layers of short vertically elongated cells, which are extremely compact and very rich in chloroplasts. In some leaves there was no distinct palisade parenchyma and the cells in this region did not differ from the other mesophyll cells except in being more compactly arranged and having a larger number of chloroplasts. The absence of palisade from such leaves may be attributed to their having been collected from more shaded sides of the plant. The rest of the mesophyll consists of ordinary spongy parenchyma with large intercellular spaces. Druses of calcium oxalate are occasionally found just below the upper epidermis. The lower epidermis is similar to the upper but its cells are much smaller and are interrupted by stomata (Fig. 14). Fig. 13 shows a diagram of a c.s. of the leaf through the midrib region.

ACKNOWLEDGEMENT

SUMMARY

1. The stele in the young stem normally consists of two rings of five bundles each. In exceptional cases the outer ring may have up to seven bundles and the inner only 3 or 4 bundles. A continuous wavy ring of sclerenchymatous cells is present around the bundles.

2. All the bundles, whether young or old, are devoid of internal phloem.

3. The cork cambium, which usually arises underneath the collenchyma may sometimes develop in its second layer at the angles and just below the epidermis at other places.

4. In older stems the periderm consists of 3–4 concentric and alternating layers of thick and thin-walled cork.

5. The pericyclic sclerenchyma breaks up into separate bands bridged by stone cells that are secondarily differentiated. Some of these bands are thrust out and eliminated by the development of a cork tissue below them.

6. As secondary growth advances, the vascular bundles are radially split by sheets of secondary vascular rays so that an old stem contains numerous separate bundles instead of the original ten.

7. The leaf and petiole also lack internal phloem and the bundles are of the ordinary collateral type. Crystals of calcium oxalate are occasionally present.

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5. Jeffrey, E. C. (1917) .. *The anatomy of woody plants,* Chicago.

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XYLEM RAYS IN RELATION TO THE ECCENTRIC RINGS AND THE SPIRAL GRAIN IN THE WOOD OF *PINUS LONGIFOLIA*, ROXB.

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Xylem rays, or *wood rays*, also known as *medullary rays*, or *pith rays* are radiating strips of cells running centrifugally through the xylem and also extending through the phloem as *phloem rays*. The rays are initiated by the cambium, and once formed, are increased in length indefinitely by the cambium. In conifers, they are generally one row of cells thick; in pines, wider rays are also found containing in the middle a horizontal resin duct. They are from one to about 16 cells in height. As they run radially outwards through the secondary xylem they gradually run apart or diverge from each other, but more and more rays appear from the cambium at later stages in between the older diverging rays, as the circumference of the vascular cylinder rapidly increases with the addition of new secondary tissues (1, 2). In the transverse section of a normal stem where the growth rings are concentric, the rays appear like innumerable radii running almost straight from the centre towards the circumference.

In horizontal branches of conifers the growth rings are wider in the lower part than in the upper part of the branch; *i.e.*, the subsequent growth rings are eccentric having their wider region almost always on the vertically lower side of the branch. In the transverse section of such a branch, the author has observed that the rays are straight in the antero-posterior plane of the branch, but in its lateral plane the rays appear to be curving upwards (Text-fig. 1). In such coniferous branches, we may suppose that the centre of the gradually widening successive growth rings goes on changing its position gradually downwards in a vertical line corresponding to the antero-posterior axis of the branch. The tendency of the growth of a cambial cell being always in a radial line upto the circumference forming a normal to it, the strip of ray in an outer eccentric ring makes an angle with the strip of the inner ring with which it is continuous (Text-fig. 2).

In the main axis of the twisted chir pine (*Pinus longifolia*) there are eccentric growth rings and the widest parts of all such rings may be on different radii in a transverse plane. In Plate XIII, Fig. 7, showing the transverse section of the stem of a twisted pine, the eccentricity of the successive rings, as also indicated by the compression wood, lie on different radii. The centres of these rings do not lie in one line as in the case of a horizontal branch. Consequently the rays are observed to pass out in a zigzag course

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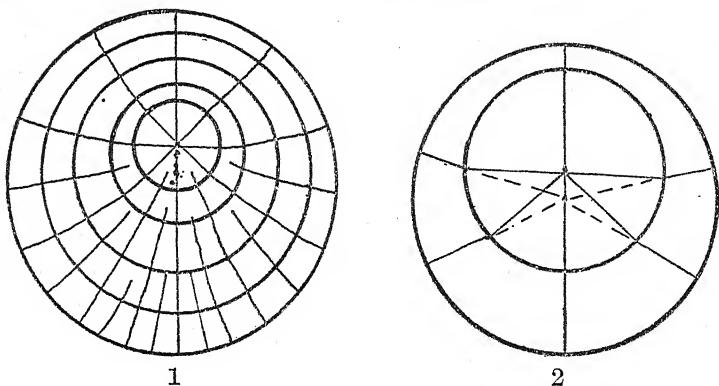
Text-figs. 1–2.—Fig. 1. Diagrammatic representation of the annual rings and of the wood rays in the transverse section of a coniferous branch. Fig. 2. Explaining the curving of rays in the above, the radius of the outer eccentric ring forms an angle with the radius of the inner ring.

forming normals to the circumferences of these eccentric growth rings.

In the twisted chir pine the degree of twist of the spiral grain goes on changing from year to year, sometimes increasing and sometimes decreasing (3). As the spiral grain, both in its production and in its direction, has been found to be correlated with the eccentric growth rings and with the change of sides of the eccentricity in these rings at different height levels (4), it was considered worthwhile to study whether the zigzag course of the wood rays has got any relationship with the degree of twist from year to year.

Consequently, a transverse section of a small sector of a 39-year old stem of a twisted chir pine showing externally 30 degrees of left twist, was taken to show the course of the wood rays from the 4th to the 39th ring and photomicrographed in six plates (Plate XIII, Figs. 1 to 6). Tangential longitudinal sections from outside through most of the annual rings of the same sector have been studied and the degree of inclination of the fibres or tracheids has been calculated in relation to a vertical side. Such a section is shown in Plate XIII, Fig. 8. The following table shows the degree of inclination of tracheids in some of the different annual rings.

Annual Ring	Inclination of Tracheids in degrees	Annual Ring	Inclination of Tracheids in degrees
39	30	19	30
37	30	17	27·5
36	30	14	27·5
35	30	13	25
33	28	11 (late)	23
32	29	10	20
30	31	9	20
22	30	4	11

An attempt may then be made to correlate the degree of inclination of the tracheids with the straight or zigzag course of the wood rays, in the different growth rings.

In the 4th ring the inclination of tracheids was 11 degrees clockwise, but it went on increasing upto the 9th ring to 20 degrees and this increase may be correlated with the right-hand (clockwise) shifting or curving of the rays through the 5th, 7th and the 8th rings (Plate XIII, Figs. 1 and 2). In the 9th and 10th rings the rays are comparatively straight (Pl. XIII, Fig. 3) and the inclination remained at 20 degrees in these rings. Towards the end of the 10th ring and in the 11th ring, there is again a right-hand shifting of the rays, which might be correlated with the increase of fibre inclination to 23 degrees at the end of the 11th ring. The right-hand shifting of the rays continues through the 12th and the 13th rings (Plate XIII, Figs. 3 and 4) and is probably responsible for the increase of inclination to 25 degrees in the 13th ring and to 27·5 degrees in the 14th ring. The rays are comparatively straight through the 14th, 15th and 16th rings (Pl. XIII, Fig. 4), and the inclination of the tracheids remained at 27·5 degrees through the 14th, 15th and 16th rings upto the 17th ring. There is again a right-hand shifting of the rays in the 17th and 18th rings (Plate XIII, Fig. 5), and the inclination increased upto 30 degrees in the 19th ring. From the 20th to the 30th rings, which are very small, the inclination increased from 30 to 31 degrees and the rays are fairly straight in the region excepting in the 24th ring where there is a slight right-hand shift (Plate XIII, Fig. 5), which might be responsible for the slight rise of one degree. At the beginning of the 32nd ring and through the 33rd ring there is a reverse shifting, *i.e.*, a left-hand or anti-clockwise shifting of the rays (Pl. XIII, Fig. 6). This may be correlated with the decrease in the inclination of tracheids to 29 degrees in the 32nd ring and to 28 degrees in the 33rd ring. In the late wood of the 33rd ring, a few of the rays have tended to straighten and in the 34th ring, all the rays have straightened. So in the 35th ring, the inclination of tracheids is found again to be 30 degrees. The constant inclination of 30 degrees from the 35th to the 39th ring may be correlated with the almost straight course of the rays through these rings.

SUMMARY

In horizontal coniferous branches, where the successive growth rings are eccentric but the widest regions are confined to the vertically lower side of the branch, the wood rays are found to be curving upwards, on the lateral sides of the branch. This may be explained by assuming that the rays form normals to the circumferences of the successive rings.

In the stem of the twisted chir pine, the growth rings are eccentric and the widest regions of the different rings may lie on different radii. The rays are observed to take a zigzag course, if the

eccentricity changes sides very often, forming normals to the circumferences of the successive rings.

The deviation of the wood rays from its straight course through the wood, seems to be correlated with the appearance of spiral grain and its clockwise deviation tends to produce the left twist (clockwise course upwards) of fibres. Further clockwise deviation in the course of the wood rays tends to increase the left twist of the fibres and the anit-clockwise deviation of the rays tends to decrease the left twist.

The author takes this opportunity to thank Mr. G. V. Chalam, M.SC., for the preparation of the photomicrographs for this paper.

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EXPLANATION OF PLATE XIII

FIGS. 1–6. Photomicrographs of the transverse section of a small sector of a 39-year old stem of a twisted chir pine showing all the rings from Ring 4 to Ring 39, which are numbered on the left-hand side. The courses of the medullary rays are indicated by the innumerable dark lines.

FIG. 7. Transverse section of the stem of a twisted chir pine, showing eccentric rings with dark compression wood, lying on different radii. The rays have taken a zigzag course to form normals to each circumference.

FIG. 8. Tangential longitudinal section of the wood of the above sector of the 39-year old stem showing inclination of tracheids in relation to a vertical side of the sector.
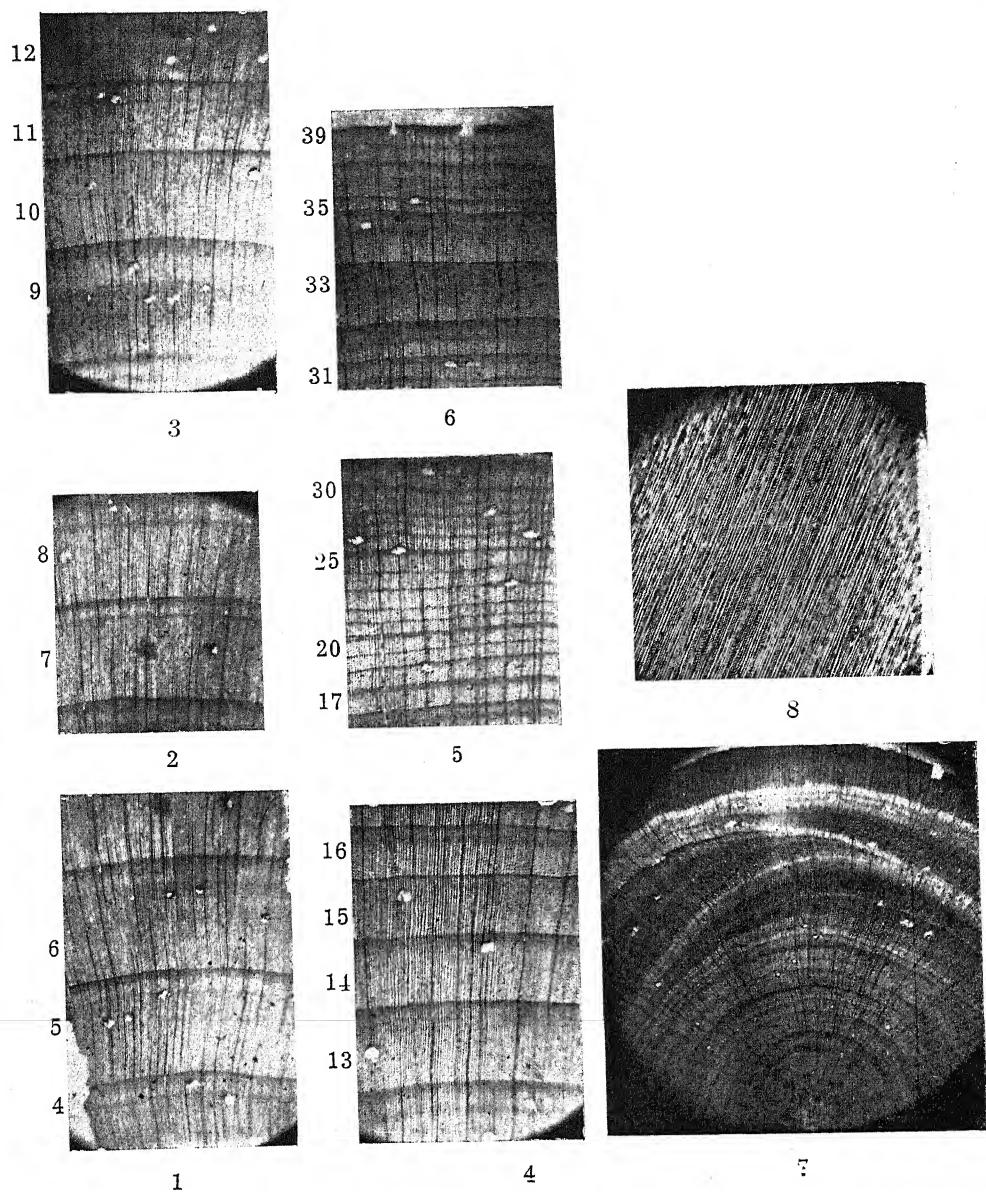
PARASURAM MISRA—

XYLEM RAYS IN RELATION TO THE ECCENTRIC RINGS AND THE SPIRAL GRAIN IN THE WOOD OF PINUS LONGIFOLIA

STRUCTURE OF THE GYNŒCIUM IN MALE FLOWERS OF *OSMANTHUS SUAVIS* KING

By A. C. Joshi

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Osmanthus Lour. (Fam. Oleaceæ) is a small genus of evergreen shrubs or small trees with less than a dozen species, but distributed from N.W. Himalayas through China and Japan to Western N. America. Two species, *O. fragrans* Lour. and *O. suavis* King, are found in India, the former in temperate Himalayas from Garhwal to Sikkim and Khasia Hills at a height of 4,000—7,000 ft., the latter in subalpine Himalayas of Nepal, Sikkim and Bhotan at a height of 9,000–10,000 ft. The calyx and corolla in all species are tetramerous, stamens dimerous, and gynœcium consists of a 2-celled superior ovary, with two ovules hanging from the apex in each cell. The flowers, however, vary a great deal in their sexual expression. They may be hermaphrodite, polygamous, monœcious or diœcious. *Osmanthus suavis* possesses flowers of two kinds, hermaphrodite and male. The latter have all the parts, but the gynœcium is rudimentary and sterile. Even then the gynœcium shows several features, which appear to be of considerable interest with reference to the morphology of the carpel. Therefore, an attempt has been made in the present paper to describe the anatomy of the male flowers of this species with special reference to the form and the vascular structure of the gynœcium.

The investigation is based on the study of serial microtome transverse sections of flowers obtained from a tree growing at the Llyod Botanic Garden, Darjeeling. The material was collected and sent to me by the Curator of the Garden, and I take this opportunity to express my indebtedness to him for the same. I also desire to thank here Dr. S. K. Mukerji, Curator of the Herbarium, Royal Botanic Garden, Sibpur, Calcutta, for confirming the identification of the material. It has so happened that all the material sent to me consists of male flowers. I had thus no opportunity so far to study the comparative structure of hermaphrodite flowers, in which the gynœcium is fully developed. The work is thus somewhat incomplete, but the results even as they are warrant publication on account of their general interest and the light they shed on the morphology of the carpel:

Out of the male flowers available to me, I have cut four flowers, one comparatively young with anthers only at the microsporemother cell stage, the other three nearly fully developed and possessing quite mature pollen grains. Three flowers, the young one

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and two old ones, showed an essentially similar structure, while the fourth flower differed from the rest in showing an extra carpel.

STRUCTURE OF THE NORMAL FLOWERS

The pedicel shows quite normal structure. The vascular bundles are arranged in a compact ring. There are no medullary bundles as seen in several species of *Jasminum* (Joshi and Fotidar, 1940) or any other anomalous features. The vascular supply of the calyx consists of eight traces, four midrib bundles alternating with four commisural bundles. The latter further up branch into two each and form the marginal veins of the sepals. Each calyx lobe thus gets three bundles. The stele of the floral receptacle soon after the departure of the calyx traces gives out six vascular strands, which are larger than all the rest. Four of them occupy a diagonal position and are the petal traces. The other two placed laterally are the stamen traces. These facts are illustrated by Figs. 1 *a–b* and 3 *a–b*. Figs. 3 *a–b* are more clear, as in Fig. 1 the flower is cut somewhat obliquely. Each petal trace in the corolla-tube splits into three bundles, while the stamen trace runs as such through its entire course in the corolla-tube till it enters the base of the subsessile stamens.

The central stele after the departure of the calyx, corolla and stamen traces resolves into six bundles, arranged in two groups of three each. One group is on the anterior, the other on the posterior side of the flower. These are the vascular traces of the gynœcium, each group supplying one carpel. The median bundle of each group is the dorsal trace and runs along the midrib or dorsal suture of the carpels, while the lateral bundles form the marginal veins of the carpels that run along the ventral sutures. The three main veins of each carpel frequently branch. Therefore the number of bundles in a transverse section of the ovary is often seen to be more than six (Fig. 1 *b*), but such branching is confined only to the base of the ovary and higher up the extra bundles disappear (Fig. 1 *c*). Many peculiarities are observed in the ovary which, as judged from the previous descriptions, have not been noted in the bisexual flowers, where the gynœcium is fully developed and fertile. The ovary is never completely bilocular. The ventral sutures are close together for a very short distance at the base of the ovary (Fig. 1 *b*), and the ovary looks bilocular, but even here the boundaries of the two sutures are quite distinct. The ventral sutures immediately higher up move apart and only one common loculus is seen in the ovary (Fig. 1 *c*). Further, the ovary is not closed at the top. It opens out into two leaf-like open carpels (Fig. 1 *d*). The lateral carpel traces at this level have died out and each carpel shows only the midrib bundle. The form and position of the two carpels with respect to each other varies slightly in different flowers. They may be quite separate from each other as in Figs. 1 *d–f*, or they may fit into each other as in Fig. 2. When free from each other, each carpel has either a conduplicate form or is rolled over from one edge to the other.
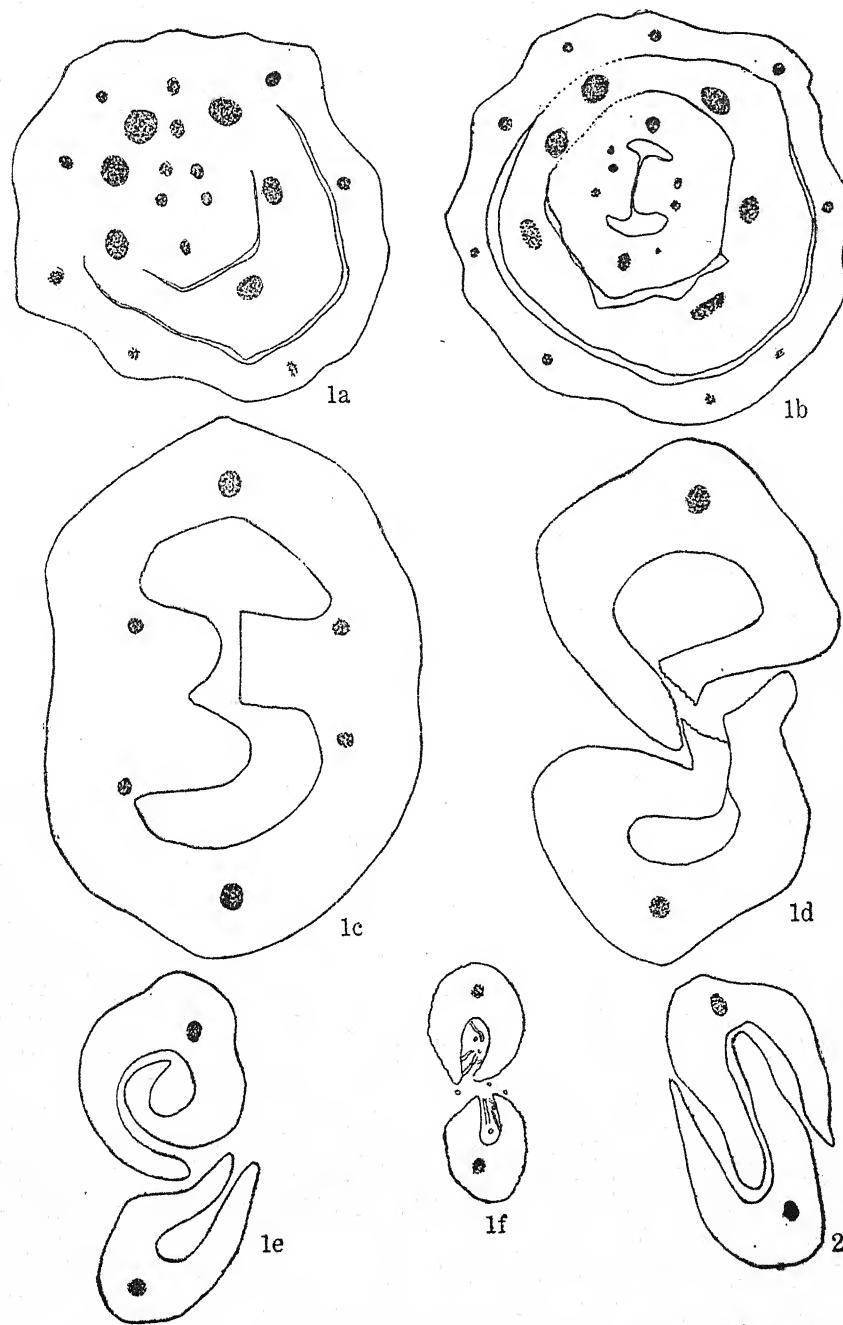
Figs. 1 and 2. *Osmanthus suavis.*—Figs. 1 *a–f*. A series of transverse sections, slightly oblique in figs. *a* and *b*, from the base upwards of a normal male flower showing the structure of the flower and the vascular supply of the different parts. The gynœcium alone is shown in figs. *c–f*. Fig. 2. Transverse section of the upper part of the gynœcium of another male flower. For further explanation see text. Figs. 1 *a* and *b*, ×45; the rest, ×85.

The anterior carpel in Fig. 1 e shows the first form, the posterior the latter form. The two open carpels in Fig. 2 show an half-equitant arrangement. There is no style or stigma. Each carpel in all cases near the apex is covered with long unicellular hairs arising from the inner surface (Fig. 1 f). These hairs perhaps represent the transmitting tissue of fertile carpels. Ovules are totally absent. There are even no rudiments of them either in the lower closed part of the ovary or higher up along the free margins of the open carpels. Another feature deserving special notice here is the comparative size and behaviour of the dorsal and ventral traces of the carpels. Generally among flowering plants the dorsal bundle of a carpel is comparatively weak. The ventral bundles are larger in size and more abundantly branched. Here however the dorsal bundle is generally better developed than the ventral bundles. It is larger in size and extends through the whole length of the carpels, while the ventral bundles are not only smaller in size but also die out before the ovary opens out into free leaf-like carpels. This fact is clear in all figures.

The histological structure of the vascular traces for the different whorls varies considerably. The calyx traces are collateral throughout their length. The corolla traces are concentric at their base, with xylem in the centre surrounded by phloem on all sides, but after they have branched, the concentric structure is lost and the vascular bundles in the corolla become collateral. The stamen traces are concentirc as they originate from the stele of the thalamus and they retain this structure throughout their length, i.e., both in the corolla-tube, filament and the connective of the anther. The carpel traces are collateral. Such histological structure of the traces for the different whorls appears to be common to other Oleaceæ. It is seen in *Olea fragrans* (Joshi and Fotidar, 1941) and *Nyctanthes arbor-tristis* (Fotidar, 1942).

Structure of the Flower with Three Carpels

The form and vascular supply of the calyx, corolla and stamens of this flower agrees completely with that of the normal flowers described above (Figs. 3 d–b). Similarly the structure and vascular supply of the two outer carpels of this flower exactly resembles that of the two carpels of normal flowers. The difference lies only in the fact that the floral axis in this case does not come to end after the formation of the whorl of two carpels. It is slightly prolonged above them, and its stele after giving off the vascular supply of the two outer carpels aggregates to form another collateral bundle situated in a plane at right angles to that of the two outer carpels (Figs. 3 a–b). This bundle higher up passes into another open carpel contained within the two outer carpels (Figs. 3 c–g). In the lower part of the gynœcium, where the ovary formed by the outer carpels is closed on the sides, this carpel alternates in position just like its vascular supply with the outer whorl of carpels (Figs. 3 d–e), but in the upper part, where all the carpels are quite free, it is enclosed by one of the outer carpels. This
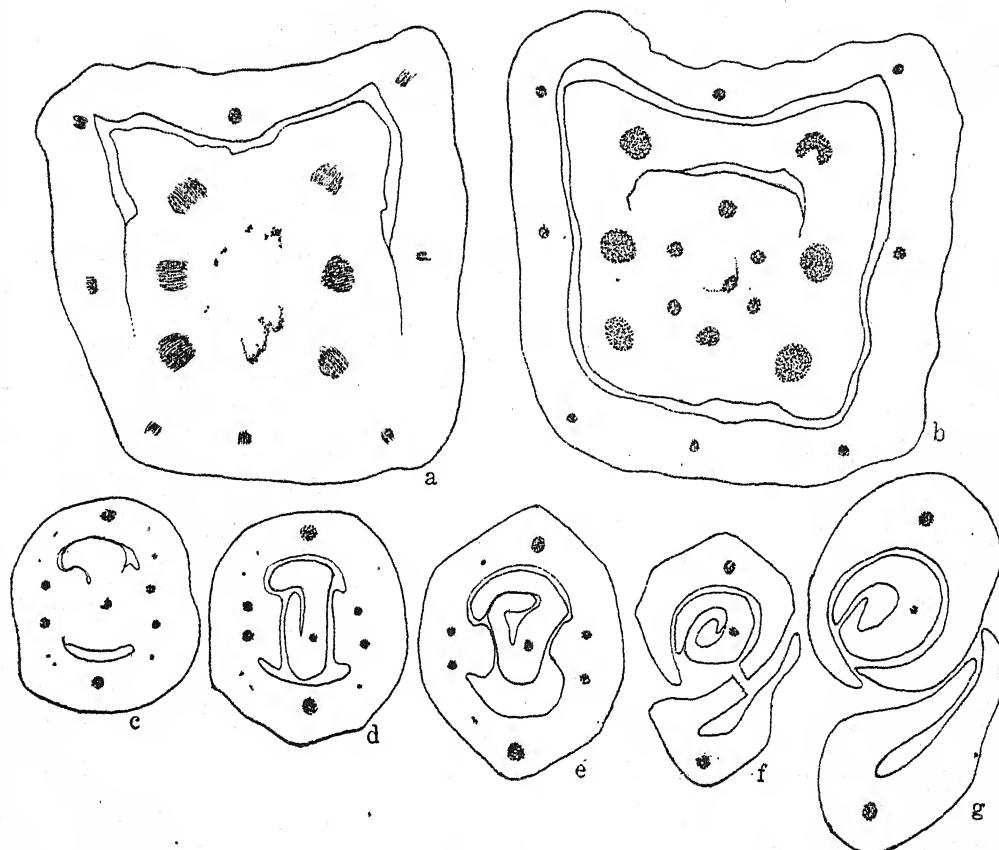
Fig. 3 *a–g*. *Osmanthus suavis*.—A series of transverse sections from the base upwards of a male flower with three carpels showing the structure of the flower and the vascular supply of the different parts. The gynœcium alone is shown in figs. *c–g*. For further explanation see text. ×85.

extra carpel throughout its length possesses one vascular bundle, which never branches.

DISCUSSION

The gynœcium of the Oleaceæ is generally described as consisting of two united carpels occupying a median plane, but Saunders (1939) applying her theory of carpel polymorphism to this family considers, citing *Syringa*, *Forsythia*, *Ligustrum* and *Fraxinus* as examples, that the gynœcium consists of two median sterile carpels alternating with the stamens when the androecium is dimerous and two lateral fertile carpels. Rarely, according to her, the gynœcium consists of 4 + 4 carpels, as in the female flower of *Osmanthus*. She gives no special reasons for these views of hers, but we can guess that these are the same on which her theory of carpel poly-

morphism in general is based. Still it is not quite clear even on this assumption why she regards the gynœcium of *Osmanthus* as composed of 4 + 4 carpels. Comparing the structure of the gynœcium of the male flower of *Osmanthus suavis* with that of *Syringa* as described by Eames (1931), the two are seen to be built on the same vascular plan. We can then take it that she would give a 2 + 2 formula for the gynœcium of the male flower of this species. The breaking up of the gynœcium in this case in the upper part into two leaf-like open carpels, however, clearly demonstrates that it is composed of only two carpels as conceived by the monomorphic view.

A typical angiospermous carpel possesses generally three veins, the median or the dorsal bundle and two lateral or ventral bundles. The dorsal bundle is generally little branched, while the lateral bundles are greatly developed and much branched. This has been stressed as a strong point of difference between the carpel and vegetative leaves, and it has been consequently stated that the two are not homologous. Thomas (1931) on this ground has stated that a carpel is not a simple structure, but is composed of three parts. The poor development of the lateral traces in the carpels of the male flower of *Osmanthus suavis*, which is obviously related to their sterility, clearly shows that the great development of lateral traces in ordinary carpels is a purely physiological phenomenon. It is, as I stated before (Joshi, 1935), related to the fact that the lateral veins have to supply nourishment to the ovules and later on to the developing seeds, while the midrib bundle has no particular function. It has no morphological significance.

SUMMARY

The vascular anatomy of the male flowers of *Osmanthus suavis* King is described, with special reference to the form and structure of the gynœcium. The 4-lobed calyx is supplied by four midrib and four commisural collateral bundles, the vascular supply of each sepal being built on the $\frac{1}{2} + 1 + \frac{1}{2}$ plan. Each of the four petals receives a single concentric trace, which splits into three collateral bundles. Each stamen receives one bundle, which has a concentric structure throughout its length. The carpels are united in the lower part to form a closed ovary, but this is unilocular unlike the ovary of the bisexual flowers. Further, in the upper part of the gynœcium the ovary opens out into two leaf-like carpels with free margins. This clearly shows that the gynœcium is composed of two carpels and refutes the polymorphic interpretation of Saunders. There are no ovules or their rudiments. Each carpel receives three collateral traces, of which the dorsal one is better developed. The laterals are weak and die out in the free part of the carpels. This proves that the great development of the lateral veins in ordinary fertile angiospermous carpels is a purely physiological phenomenon connected with the nourishment of the ovules and has no morphological significance. A third

carpel has been observed in one flower within the two normal carpels.

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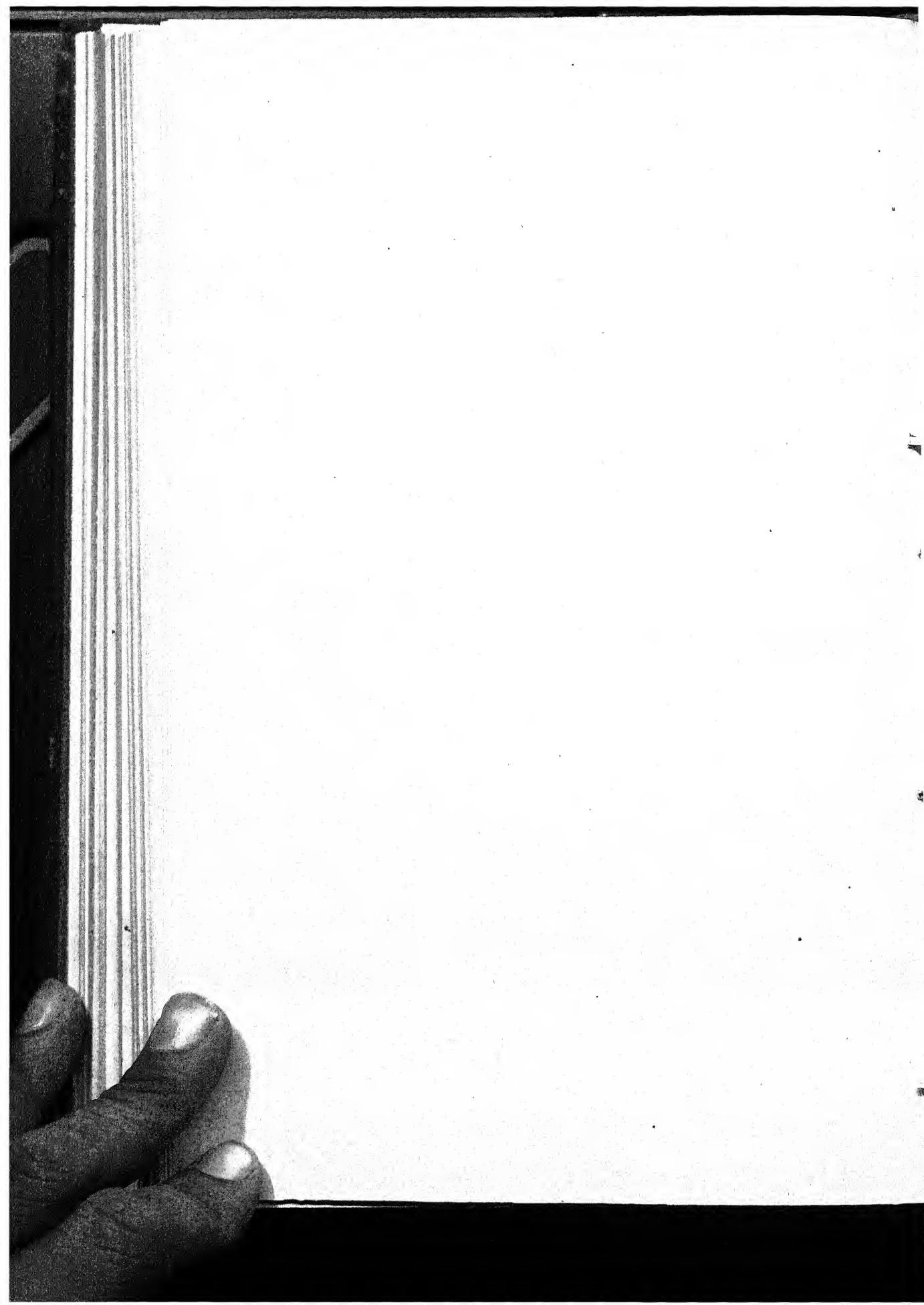
AN ANATOMICAL STUDY OF THE SHEDDING
AND NON-SHEDDING CHARACTERS
IN THE GENUS *ORYZA*

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(Communicated by P. Parija)

Shedding of the spikelets is a very common feature in the wild paddy. During the months of November and December a very interesting spectacle is presented by the naked panicles and the bare pedicels of wild paddy (Plate XIV). On the other hand during the same months cultivated paddies give quite a contrasting view with their ears fully laden with the spikelets. In the wild paddies as observed in this part of the country the grains are shed when they reach the stage of ripening. From the stage of the hardening of the grain, any slight contact would be enough to drop the grain from the axis of the pedicel. Naturally as the spikelet of the wild paddy consists of a long awn with bristles (Fig. 4), it is easily dislocated from its axis by wind or any other contact. On the other hand in the case of the cultivated paddies a certain amount of mechanical force is required to separate the grain from the ear (which is otherwise known as thrashing). From the cultivators' point of view non-shedding with the possibility of separation with a not too strong mechanical force is a very desirable, economic character.

A morphological and anatomical study of the various parts connected with this character, is made in this paper. The present investigation is confined to two shedding types namely *Oryza sativa* Var. *fatua* and *Oryza coarctata* (Roxb.), and one non-shedding type of *Oryza sativa* Var. Cuttack No. 3. Ramiah[5] states that even in the cultivated *Oryza sativa*, shattering is a varietal character and that this character may vary quantitatively, in different varieties. In the present case a typical non-shedding variety was taken in contrast to the other extremity in the wild species. All these species were grown in pot cultures in the Botanical Garden of the Ravenshaw College. In *Oryza sativa* and *Oryza fatua* the pedicel is connected to the spikelet at its base on the side of the superior palea. As such the joint of the pedicel and the spikelet is exterior in position to the rachis of the panicle. To elucidate the nature of the joint, the shape and anatomy of the joint were studied at three stages of the development of the spikelet. In the case of the shedding species, it was not possible to study

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the joint intact with the spikelet and the pedicel after maturity. It was found that in both *Oryza sativa fatua* and *Oryza coarctata* all attempts to dissect the joint for fixing, resulted in the separation of the two. Spikelets from the panicle while they were still in sheath and at the stage of slight maturity, *i.e.*, when the grain was not fully formed were taken and fixed in all the three species. For the third stage, *i.e.*, ripening stage, joints could be dissected from *Oryza sativa* without difficulty while in the other two species only pedicels after the shedding of the grain were fixed. The material offered considerable difficulty in cutting. Cedar-wood oil was used as a clearing agent to soften the tissues to some extent, and for embedding paraffin M.P. 52° C. was employed.

SHAPE OF THE JOINT

Ramiah[5] points out that shattering is dependent upon the nature of attachment of the grain in the panicle. Further, he suggests that the depression at the base of the spikelet is very slight in the shattering variety and the depression is a little deeper in the non-shattering varieties. A closer anatomical study of the joint has revealed another important factor which seems to play a great part in the shattering character.

Oryza sativa.—The pedicel is rounded off at the connective end which fits into the concavity of the base of the spikelet on the side of the superior palea (Fig. 3). In the longitudinal section this gives an appearance more like that of a ball and socket joint (Fig. 14).

Oryza sativa Var. *fatua.*—The joint appears like two discs one supported upon the other (Fig. 15). The connective end of the pedicel looks like a flat disc supporting the spikelet at its base though inclined at an angle (Figs. 4 and 5). The spikelet in its young and immature stages probably receives certain amount of mechanical support from the two bract-like out-growths at the base of the connective (Fig. 15.). The bract-like out-growths are conspicuoulsy large in this species when compared to the non-shedding species, where they are almost absent.

ORYZA COARCTATA

The joint in this species is entirely different from the above two species. From the shedding point of view, though this species is not so early as *Oryza fatua* all the same the grains are shed down as soon as they reach the stage of early ripening and the bare pedicels only are found (Plate XIV). The pedicle is connected to the spikelet vertically without making any bend. It may be seen from Figs. 16 and 17, that the connective end of the pedicel is inclined at an angle in the case of *Oryza fatua* and *Oryza sativa*. As such the vessels and other tissues make inward bend at the place where they enter into the connective end. This may account for the inclined nature of the joint, in the above two cases. On the other hand in *Oryza coarctata* the pedicel at its connective
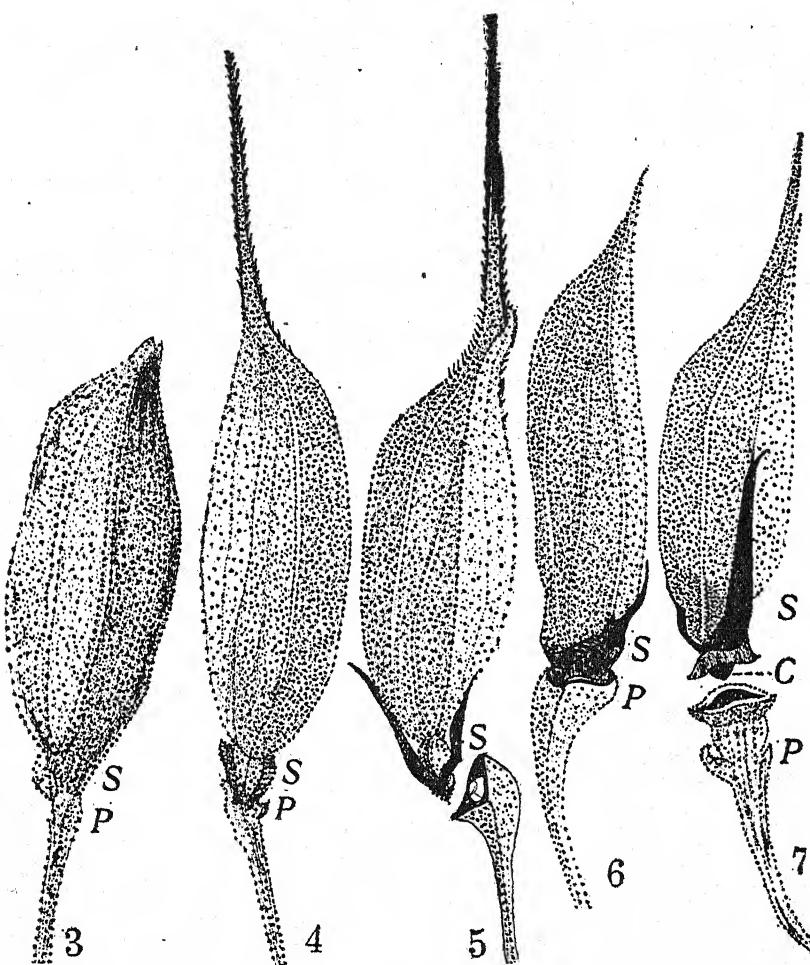
Text-figs. 3–7.—Fig. 3. Spikelet with the pedicel in *O. sativa*. Fig. 4. Spikelet of *O. fatua* at the early stages. Fig. 5. The same after ripening when the pedicel separates from the spikelet. Fig. 6. Spikelet of *O. coarctata* in the early stage. Fig. 7. The same after ripening when the pedicel separates from the spikelet.

end does not make any bend and all the tissues are more or less in straight regular rows (Fig. 18). The surface of the pedicel looks like a cup with a central cavity. The small peg-like structure protruding at the base of the spikelet fits into this concavity while the surrounding rim fuses with the base of the spikelet. (Figs. 6 and 7).

Relative measurements of the connectives of the pedicel in the joint are given in the table below:—

12a

			Oryza sativa mm.	Oryza fatua mm.
Height	..	..	0·14	0·035
Breadth	..	..	0·28	0·28

It is evident from the above measurements that in *Oryza sativa* the connective of the pedicel makes a deeper penetration into the spikelet than that of *Oryza fatua*. It is as much as four times that of *Oryza fatua*. Further as stated earlier the end of this connective rounds off inside the spikelet making more or less a ball and socket joint (Fig. 9). The diameter of the connective in both the cases is the same but the pedicel of the *Oryza fatua* looks like a flat disc without any depth of penetration into the spikelet. In the case of *Oryza coarctata*, it is quite different where the vascular tissue and its surrounding tissues form a peg-like structure which is about 0·42 mm. in height. This peg is broader in the beginning and narrows down to the end (0·245 mm. to 0·0525 mm.). This peg fits into the concavity of the pedicel while the base of the entire spikelet rests on the widened surface of the pedicel (Fig. 6).

ABSCISSION LAYER

The phenomenon of the abscission layer in leaves, buds and fruits has been extensively investigated in its anatomical and chemical aspects. von Mohl[7] was the first to discover the formation of a definite separation layer before the fall of a leaf. Wiesner[7] confirming the observations of von Mohl formulated the theory that the intercellular substances of the cells of the separation layer are dissolved by the action of the organic acids developed in the leaves. Mangin's[7] finding about the pectic nature of the middle lamella or cell walls in plants brought forth the theory that the abscission process is caused by the dissolution of the pectose and the calcium pectate of the middle lamella.

Lee[3] reported the disappearance of the middle lamella only, which was confirmed by Hannig[2] in the case of flowers also with the exception of *Mirabilis Jalapa*, where the enitre cell wall was reported to have disappeared. Later on Tison[7] also stated as supported by Lee also that in general the secondary membrane of cell walls alter and disappear leaving only thin tertiary membrane lining the cell lumen. Lloyd[4] contradicting the observation of Hannig about the disappearance of the entire cell wall that the greatest degree of alteration takes place by hydrolysis in the cells of the separation layer where a complete digestion of the part of the primary and secondary walls takes place. Consequently the tertiary walls are extenuated separating from each other. Further mechanical resistance of the older parts appear to influence the

plane of separation and the xylem tubes which are not included in the separation layer, are ruptured mechanically. Starch in the cells of the separating layer and the adjoining tissues happens to be the source of energy for the separation cells during their growth. Lloyd asserts further that neither the cytoplasm nor the nuclei display any degeneration changes which on the other hand are quite alive and bear evidence of greater physiological activity when the separation is achieved.

Yoshito Yamasaki[8] studying the abscission of the spikelet in *Oryza sativa* finds that the separation is due to abscission tissue in the part of the spikelet between the empty glume and the glume rudiment.

Yoshio Takenouchi[9] also finds a special tissue in the boundary between the grain and its supporting stalk. According to him it consists of 1–3 layers of lignified thin walled cells, which become dry when the grain begins to ripen with the natural consequence of their separation from their stalks.

In the present investigation certain broad anatomical features in the formation of the abscission layer in the two species (three types) of *Oryza* are studied and an explanation is put forth for the relative shedding and non-shedding of the grain in the above species.

Oryza sativa var. *C. No. 3.*—Very young spikelets while they are still in the sheath but just before the emergence were taken. In all the cases the topmost 20 spikelets were dissected and the joints were fixed in aceto-alcohol. Longitudinal sections of 20 μ to 30 μ were cut and stained in safranin. Abscission layer commences just below the epidermis, consisting of a narrow band of transverse cells. In the present case the band consists of one layer of cells only. These are quite distinctly observed as characterised by the smallness in their size and the dense cytoplasm. Further, this layer of cells is entirely different from the cells of the pedicel and the spikelet as marked by their very thin cell-walls. In this case the separation layer is not extended throughout the breadth of the joint. It does not extend even up to the central vascular tissue. All around the central vascular tissue about 6 to 7 layers of cells with lignified walls take a bend at right angles twice and extend into the tissues of the spikelet (Figs. 8, 9, 12), and as such the vessels in the centre happen to be protected all around by the above bands of cells.

By the time the spikelet is slightly mature separation of the cells in the abscission layer is achieved (Fig. 14). Throughout the length of the layer loose cells are found. Granular protoplast found in the previous stage could not be observed in this stage. Both from the shape and capacity for staining, the cell walls seem to have undergone a chemical change. They are now comparatively well stained and the cells are more enlarged than before. A slight unequal swelling in the cell walls was also noted. The separation of the cells may be brought about by the dissolution of
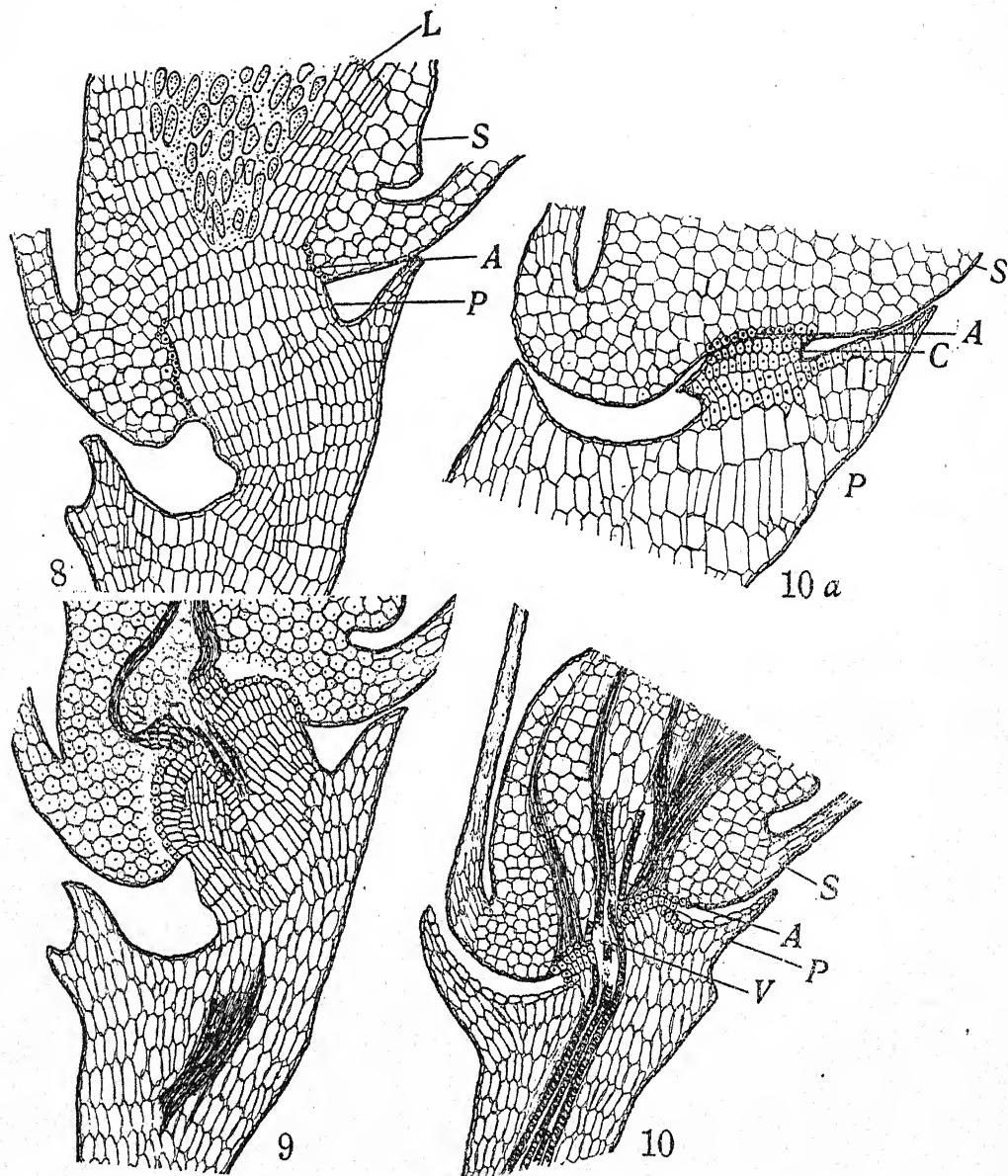
Text-figs. 8–10 a.—Fig. 8. Tangential section of the joint in *O. sativa* where two bands of cells from the pedicel entering the spikelet are shown. Fig. 9. The same in longitudinal section at the median portion showing the central vascular tissue. Fig. 10. Longitudinal section of the joint in *O. fatua* at the median portion showing the abscission zone with two layers of cells. Fig. 10 a. Joint of the same from the upper portion.

the middle lamella whose absence is quite conspicuous. The same two bands of 7 to 8 rows of lignified cells are observed protecting the central vascular tissue. These bands of cells make a double knee-shaped bend and enter into the base of the spikelet (Fig. 12). Further the epidermis of the outer glume and the pedicel remains continuous. The last stage was taken when the spikelet had completely ripened and ready for harvest. The spikelets were cut along with their pedicel and the kernels were removed by cutting them at the position of the embryo. Thus the joints were fixed along with the base of the spikelet. Since the material was found to be very hard and brittle to cut, it was kept in 2% KOH solution for 24 hours and thick longitudinal sections of 40 μ to 50 μ were cut.

At this stage continuity of the epidermis between the pedicel and the spikelet is lost (Fig. 16). There is a clear gap between the base of the spikelet and the pedicel where hitherto, the cells of the abscission layer were found (Fig. 18). Probably the cells might have been dissolved in the KOH solution during the softening treatment or might have been dropped off when the spikelet has undergone dehydration during the post-maturity stages. All the same, those bands of lignified cells on either side of the vascular tissue remain unaffected binding the spikelet to the pedicel with their double knee-shaped bend (Fig. 16).

The bands of lignified cells constitute 50% of the total number of longitudinal rows of cells in the pedicel. Consequently the continuity of the spirally thickened vessels between the pedicel and the spikelet remains unbroken, effectively protected by those bands of cells.

Oryza sativa var. *fatua.*—As stated before, though the actual shedding in this species may take place in nature when the spikelet is ripening (*i.e*, appearance of a black shade) slightest touch is enough to dislodge the grain from the pedicel, even at the stage when the kernel is fully formed but still green in colour. In this species the abscission layer is formed much earlier than in the non-shedding species as will be explained in the following observations.

In the early stages (*i.e.*, while the panicles are still in sheath) the topmost 20 spikelets were dissected and the joints were fixed. Longitudinal section were taken from the median as well as the upper portions of the joint (Figs. 10, 10 *a*, 14).

Here, unlike the case of *Oryza sativa* the separation layer commences from the epidermis (Fig. 9). One or two small cells of the above layer appear to have broken the continuity of the epidermis between the spikelet and pedicel. Further the abscission layer in this species consists of two layers of transverse cells (Fig. 10). The most improtant character which demarks this species from the cultivated one is the extension of the separation layer throughout the breadth of the pedicel. In the longitudinal section it commences from the epidermis and proceeds up to the central vascular tissue on either side (Fig. 13). No protection is offered
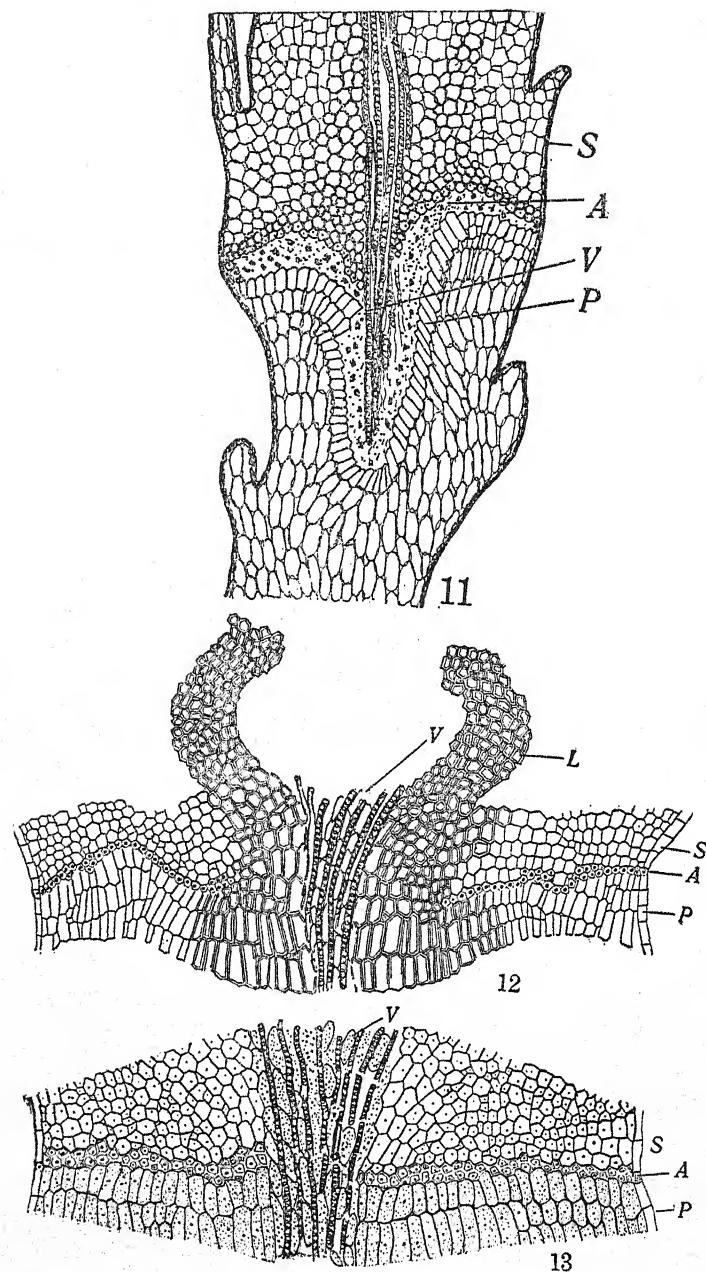
Text-figs. 11–13.—Fig. 11. Longitudinal section of the joint in *O. coarctata* showing the disintegrated cells of the abscission zone. Fig. 12. Highly magnified diagram of the median portion of the joint in *O. sativa* showing the partially formed abscission layer and the two bands of lignified cells on either side of the vascular tissue. Fig. 13. Drawn to the same magnification in *O. fatua* showing the complete formation of the abscission layer and the absence of lignified bands.
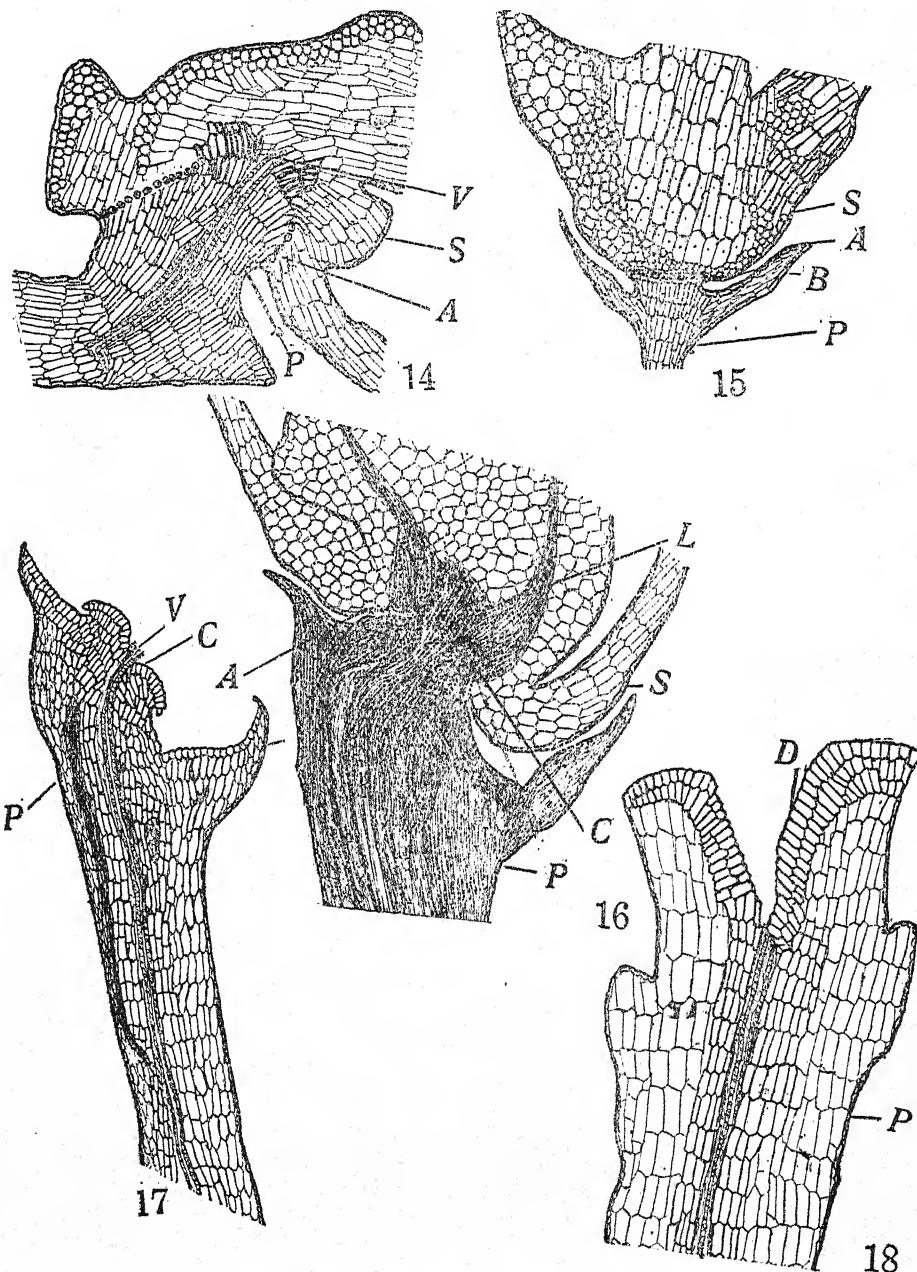
Text-figs. 14–18.—Fig. 14. L.S. of the joint in *O. sativa* at the stage of slight maturity. Fig. 15. The same in *O. fatua*. Fig. 16. L.S. of *O. sativa* at the stage of ripening. Fig. 17. L.S. of the pedicel of *O. fatua* after the shedding of the grain. Fig. 18. L.S. of the pedicel of *O. coarctata* after shedding.

A, Abscission layer; *B*, Bract-like outgrowths; *C*, Connective end of the pedicel of the spikelet; *D*, Concavity; *L*, Bands of lignified cells; *P*, Pedicel; *S*, Spikelet; *V*, Vessels.

to the central vascular tissue by any bands of lignified cells. At the same time the cells around the vascular tissue are not so lignified as in the other case and as such they easily undergo changes during abscission. Thus but for the vascular tissue the pedicel and the spikelet are separated from each other by the abscission zone consisting of two layers of cells (Fig. 15). Spikelets taken at the same stage as that of *Oryza sativa* have shown much advanced stage of the abscission layer than the observed in the non-shedding one. The protoplast in the cells of the separation layer has already begun to diminish. The cells are slightly elongated and the cell walls are well stained. The dissolution of the middle lamella also is observed here and there with the consequent separation of the cells.

Observations made in the second stage have shown that the cells in the abscission layer have distinctly separated from one another by the dissolution of the middle lamella (Fig. 15). The cells are quite apart from each other and most of them are found in a state of collapse with a thin layer only around the cell lumina, as such it may be stated that as suggested by Lloyd in *Mirabilis Jalapa*, that the abscission is caused by the complete digestion of a part of the primary and secondary walls. The vessels also are broken here and there, particularly at the place where they make a bend to the spikelet. It would appear that the spikelet is mainly supported by the vessels and the very meagerly lignified fibres around them.

In the next stage the bare pedicel after shedding of the spikelet was taken and longitudinal sections were cut (Fig. 17). Here the cells in the pedicel are lignified as those in the pedicel in the *Oryza sativa*. Further from the broken ends of the vessels, it was observed that these were broken at the same level where the abscission layer hitherto was formed. On the entire surface of the pedicel, ruptured cells were observed which happened to be the cells of the adjacent layer of the abscission zone. The cells also might have been probably effected by the activity of the above zone.

Oryza coarctata.—In this species the abscission zone commences from the epidermis takes a V-shaped bend at the centre and passes on to the epidermis on the other side (Fig. 11). The abscission zone consists of 3 to 4 layers of transverse cells and these cells are bigger than the cells of the separation layer in the other two species. These layers of abscission cells extend about 0·3 mm. towards the centre and then make a bend in the vertical plane upto a depth of 0·9 mm. In the same way from the other side also the layers of the abscission cells come and join together. The vessels are broken towards the tapering end. Finally when the spikelet sheds down, a concavity is left in the axis of the pedicel (Fig. 18).

CONCLUSION

From the above observation it may be concluded that the shedding in *Oryza* genus is caused by the formation of the abscission

layer between the pedicel and the spikelet. In the the above genus some of the cultivated paddies do not shed because the abscission layer is not fully formed. In these, bands of lignified cells all around the central vascular tissue are not acted upon the chemical reaction of the abscission cells. Further they protect the spirally thickened vessels from mechanical rupture and at the same time binding the spikelet to the pedicel with the double knee-shaped bend at the joint. Certain amount of mechanical strength also is received from the shape of the joint in *Oryza sativa* where it is something like a ball and socket joint in nature. The development of the abscission layer in the case of *Oryza fatua* and *Oryza coarctata* is much earlier, than, the incompletely formed abscission layer of the non-shedding *Oryza sativa*.

All these factors put together contribute to the earlier shedding of the grain in *Oryza sativa* var. *fatua*. The partial formation of the abscission zone facilitates easy threshing and the bands of the lignified cells around the vascular tissue keep the spikelet attached to the pedicel till the last, without shedding the grain, in the non-shedding types of *Oryza sativa*.

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EXPLANATION OF PLATES

PLATE XIV. Panicles of the three species at the stage of ripening (1) *O sativa*, (2) *O. fatua*, (3) *O. coarctata*.

PLATE XV. Photomicrograph of the joint in *Oryza fatua*.
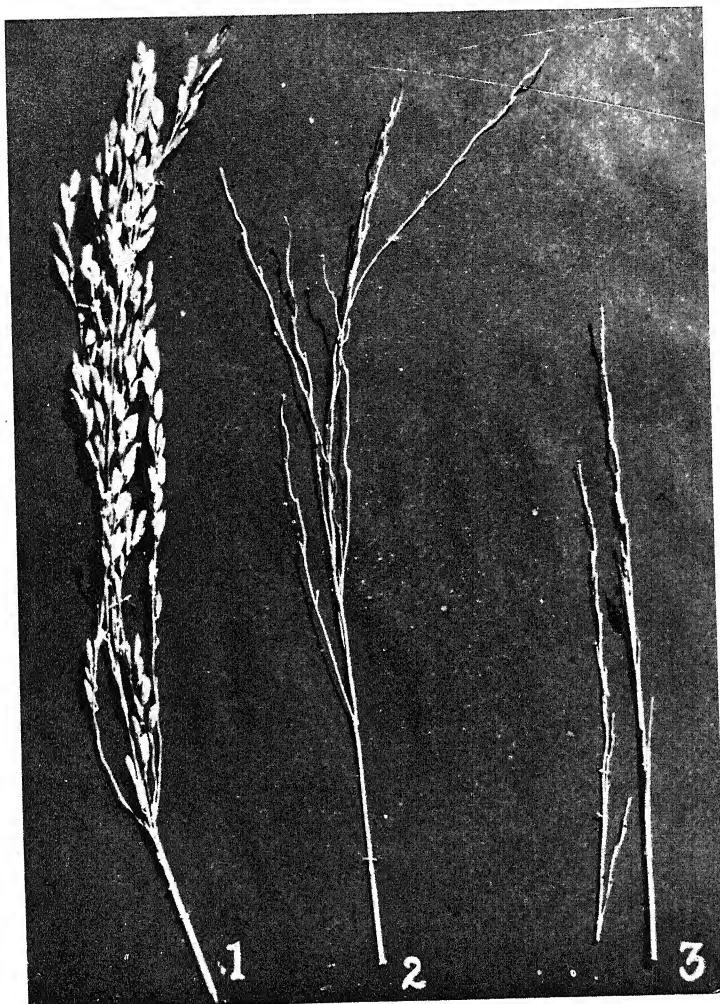
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G. V. CHALAM—

AN ANATOMICAL STUDY OF THE SHEDDING AND NON-
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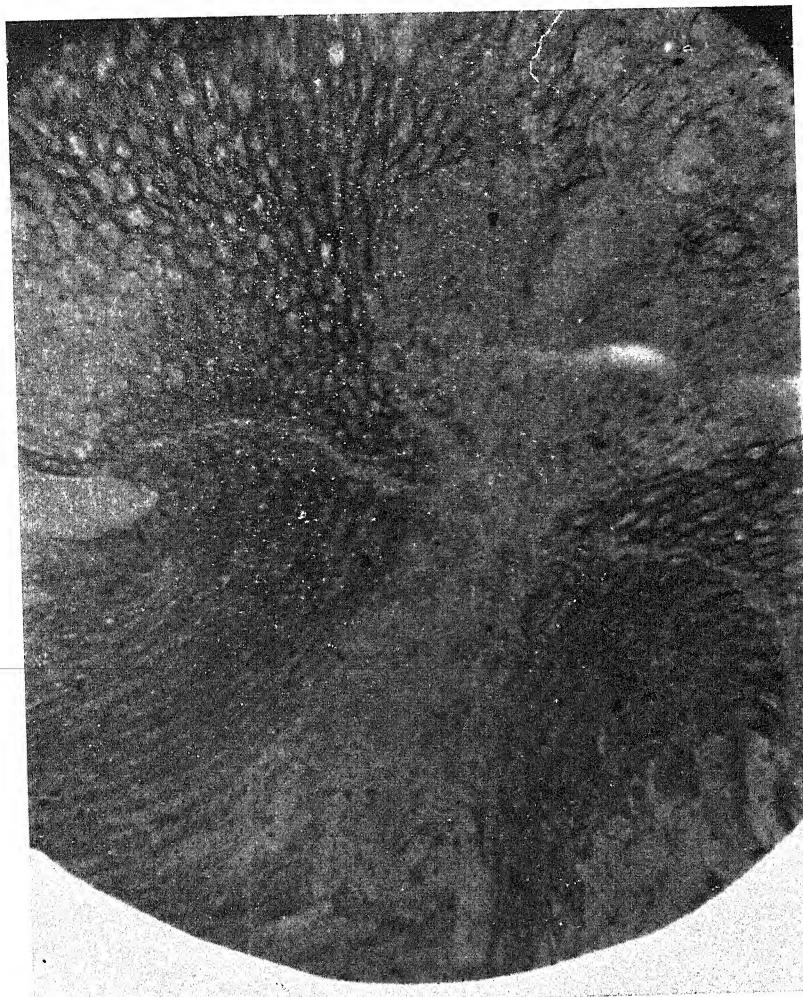
Fig. 2

G. V. CHALAM—

AN ANATOMICAL STUDY OF THE SHEDDING AND NON-SHEDDING CHARACTERS IN THE GENUS ORYZA

THE CIRCUMFERENCE-LENGTH RATIO

A NEW DIAGNOSTIC CHARACTER FOR IDENTIFYING BAMBOOS

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BAMBOO internodes increase in length from the base of the culm upwards, reaching maximum length about the middle of the stem. Most descriptions of bamboos mention a maximum and minimum length of internodes but fail to mention whether this is a variation in the longest joints of different culms, or the difference between the shortest and longest joint of the same culm. The result in that figures for internode lengths prove of little value is distinguishing one bamboo from another.

If length of joint is to have any value as a diagnostic character, measurements must always be made of similar joints in the same relative position on the culm. For instance it would be possible always to measure the fifth internode above the ground and secure figures of comparative diagnostic value, but this would not be satisfactory for several reasons. First, the lower internodes may be covered with persistent overlapping sheaths and surrounded with thick lateral branches, making measurement difficult. Second, one would have to get down on one's knees to measure the fifth joint of such bamboos as *Bambusa wamin* Br., but would have to climb a ladder to measure a similar joint of *Dinochloa distans* C.E.P. or *Tamyinwa* (Burmese) of southern Burma, in which species the internodes may reach a length of six feet.

Again it would be possible to select the longest joint of each culm for comparison, but this would entail the cutting of the bamboo and considerable checking before the longest joint could be definitely located. Whereas this procedure may be satisfactory for a forest ranger in the jungle, it would not be acceptable in a horticultural garden.

It is proposed, therefore, that the internode at $4\frac{1}{2}$ feet always be chosen for measurement. This internode has been selected as a matter of convenience: it is easy to reach, it is usually not covered by persistent overlapping sheaths, does not require the cutting of the stem, and can be instantly selected without any

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special measuring apparatus. Moreover many forestry measurements are made at this height. It is true that this joint at $4\frac{1}{2}$ feet may, in some cases, be the thirtieth from the base while in others it may be only the third, but if the measurements are taken consistently of the internode $4\frac{1}{2}$ feet above the ground, the resulting figures will have comparative diagnostic value for all kinds of bamboo.

The circumference as well as the length of this joint at $4\frac{1}{2}$ feet should be recorded. For instance the shoots of *Melocanna bambusoides* Trin. (Bamboo Garden No. A 16) two years after transplanting have joints at $4\frac{1}{2}$ feet which average $4\cdot75$ inches in circumference and 12 inches in length. These measurements may be stated in the form of a fraction, $4\cdot75/12$ which equals $\cdot40$, representing the circumference/length or C/L ratio of the internode at $4\frac{1}{2}$ feet. The C/L ratio three years after transplanting is $5\cdot87/13\cdot25$ which equals $\cdot44$.

The length of the joint at $4\frac{1}{2}$ feet of one of the commonest cultivated bamboos in the Shan States, *Mai pok mon* or *Mai mon* (Shan) also averages 12 inches, so that if the length of the internode alone were used as the diagnostic character, *M. bambusoides* Trin. and *Mai pok mon* could not be distinguished. But the circumference of the latter averages 13 inches so that the resulting C/L ratio is $13/12$ or $1\cdot08$ as contrasted with $\cdot40$ for *M. bambusoides*. This ratio has the advantage of representing both circumference and length in one figure, and of more accurately reflecting the proportions of the internode than would be possible with a figure representing one dimension only.

In taking measurements for the C/L ratio, care should be exercised to select culms from relatively mature clumps. Abnormally small stems in these clumps should not be measured but only those of average or normal size. Even with this care the C/L ratio may vary $\cdot15$ either way from the average due to the effect of age, environment, etc., on the growth of the various clumps, and to hereditary differences within the species.

After sufficient data have been obtained, the C/L ratio should prove to be of primary importance in keying out bamboos in the field; if it is to be of any use in identifying the specimens in herbaria, collectors will have to enter the C/L ratio in their field notes or on their field labels. It is not suggested that this ratio will in itself prove a positive means of identification of species, but together with other vegetative characteristics such as the number of veins per quarter inch on the leaves, the type of rhizome, the system of branching, culm sheathes, etc., it should prove a valuable vegetative diagnostic characteristic.

The C/L ratios for a number of species of bamboo are given below to illustrate the range of the figures:

Vernacular name	Place	Scientific name	C/L Ratio
Wa min (Burmese) ..	Rangoon	*Bambusa wamin* Br.	$\frac{5\cdot5}{4} = 1\cdot4$
Wa bo (Burmese) ..	do.	*Dendrocalamus gigan-teus* Mun.	$\frac{15\cdot75}{13\cdot2} = 1\cdot2$
Mai kao quai or Mai leng (Shan)	Taunggyi		$\frac{12\cdot75}{11\cdot3} = 1\cdot13$
Mai pok mon (Shan) ..	do.		$\frac{13}{12} = 1\cdot08$
Hti yo wa (Burmese)	Rangoon	*Thyrsostachys siamensis* Gam.	$\frac{7\cdot5}{8} = \cdot94$
Wa net (Burmese) ..	do.	*Bambusa vulgaris* Schrad.	$\frac{10\cdot07}{10\cdot9} = \cdot92$
Shwe wa (Burmese) ..	do.	*Bambusa vulgaris* var. *striata* Riv.	$\frac{9\cdot5}{10\cdot5} = \cdot90$
Mai shang (Shan) ..	Taunggyi	*Dendrocalamus mem-branaceus* Mun.	$\frac{8}{11\cdot5} = \cdot70$
Mai bawng or Mai mawng (Shan)	do.		$\frac{7\cdot96}{17\cdot3} = \cdot46$
Tabindaing wa (Burmese)	Rangoon	*Melocanna bambusoides* Trin.	$\frac{5\cdot87}{13\cdot25} = \cdot44$
Tin wa (Burmese) ..	do.	*Cephalostachyum per-gracile* Mun.	$\frac{5\cdot6}{16\cdot4} = \cdot35$
Tabindaing gale (Burmese)	do.	*Melocanna humilis* Kurz.	$\frac{2\cdot75}{16} = \cdot17$

SUMMARY

A new measurement for the length of bamboo internodes is proposed which is called the Circumference-Length Ratio. This measurement is to be made in all cases of internodes $4\frac{1}{2}$ feet above the ground. This ratio has the value of representing in one figure both circumference and length, and of accurately reflecting the proportions of the internode. This C/L ratio should prove to be a very useful diagnostic characteristic.

THE CHAROPHYTES OF THE BOMBAY PRESIDENCY—III

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THIS account of the Charophytes in the Bombay Presidency is in continuation of the series of papers published by the author in this *Journal* (1931, 1935, 1940). Here are recorded two more species from the southern parts of the Presidency and additional results of further explorations have been appended. The descriptions of some of the species mentioned in the past papers have been completed.

NITELLA

1. *Nitella acuminata* A. Br.—Chrac. Ind. Orient. in *Hooker's Journ. Bot.*, Vol. I, p. 292 ; A. Braun and O. Nordstedt, *Fragmente einer Monographie der Characeen*, 1882, p. 35, Taf. IV, fig. 88.

Monœcious. Stem stout, 500–800 μ thick. Branchlets 6, once forked ; dactyls unicelled, 3–4, acuminate to a sharp point. Primary rays very long. Oogonia mostly solitary, rarely geminate, 350 μ long (incl. coronula), 332 μ broad. Coronula short, 33 μ high, persistent. Convolutions 7. Oospore 250 μ in diameter. Antheridium 250 μ in diameter.

Locality.—Pond, Borivali, Bombay, Sept. 1931.

This species is a variable one. It was first collected by Stockes in 1847 from Concan, Bombay Presidency. There are two varieties, *viz.*, var. *Belangeri* which is stouter and resembles our specimen and var. *indica* a slender variety from Java. Sub-species *N. Gollmeriana* Br. differs from the present species by its smaller size and the acute dactyls. Another sub-species *N. glomerulifera* Br. differs by its segments of the sterile branchlets being longer. These sub-species described by Braun hang on very thin threads as the vegetative characters are hardly sufficient to rank them as sub-species. There is no doubt that on revision they will lose the rank. *N. flexilis* Ag. resembles *N. acuminata* in its monodactylous character but differs in the smaller size of its gametangia and non-acuminate dactyls.

2. *Nitella Annandalei* Pal.—B. P. Pal, "Burmese Charophyta," *Journ. Linn. Soc. Bot.*, 49, 1932, p. 70, Pl. 10 ; Groves, " Notes on Indian Charophyta," *Journ. Linn. Soc. Bot.*, 46, 1924.

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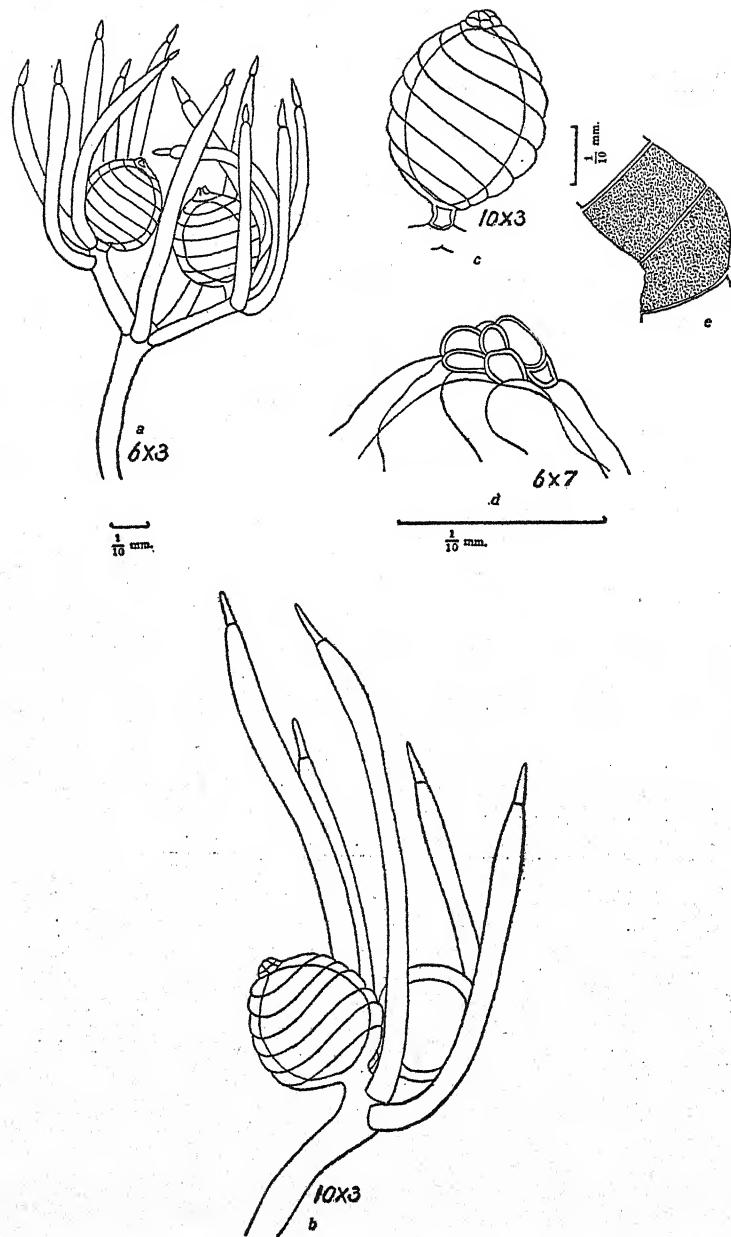
Fig. 1. *Nitella Annandalei* Pal.—(*a*) Fertile branchlet (×c.50); (*b*) Branchlet showing monœcious development (×c.70); (*c*) Oogonium (×c.70); (*d*) Coronula of the oogonium (×c.270); (*e*) Oospore membrane (×c.270).

Diœcious. Female plant.—Plant rather stiff and large. Stem slender 300–330 μ thick. Branchlets 6, two to three times furcate, secondary rays→6 of which one or two sometimes remain simple, tertiary→6, dactyls usually 6, equal, cylindrical, elongated, 2-celled, lower cell very long, end-cell small, short, acute. Sterile shoots large, long. Fruiting heads small, enveloped in mucus. Oogonia on first and second furcations, on a distinct stalk cell, 350 μ long (incl. coronula), 280 μ broad ; coronula, 17 μ high, 50 μ broad ; convolutions 7–8 ; oospore 215 μ in diameter, spherical. Membrane closely granulate.

Male plants not collected.

Locality.—Flowing rivulet, Castle Rock. December 1940.

Some of the plants collected were found to be sub-monœcious, as a few antheridia c. 250 μ in diam. were observed on the female fruiting heads. Such a condition is sometimes observed in a diœcious species *Nitella dispersa* by Groves and Allen (1927).

The plant described here resembles the species described by Pal (1932) as *N. Annandalei* and by Groves (1924) No. 4 without name but as new species. Although only the female plants were found, the dactyls were so characteristic that the plant is unlikely to be mistaken for any other. The description given here is that of the female plant which so far was not gathered by the earlier collectors. This find makes the description of the species complete in conjunction with Groves' and Pal's descriptions. However, it must be pointed out that our specimens differ from the male plant in size and the number of branchlets and rays. In order to make sure, the specimens were sent to Dr. Pal for confirmation. He expressed the opinion that he saw no reason for disagreeing with me. We are now certain of its taxonomic position among Bicellulatæ.

The species appears like *N. dualis* Nordst. (*N. superba* Pal.) but differs from it by having six dactyls, the end-cell being short and *not allantoid* but conical and the dactyls being uniformly 2-celled. It also resembles *N. dispersa* Br. and is allied to it but *N. dispersa* has branchlets four times forked, fewer rays, and unequal dactyls. It has similarities with *N. globulifera* Pal but the latter is a very small plant.

3. *Nitella batrachosperma* Br.—Braun and Nordstedt, *op. cit.,* 1882, p. 37, Taf. V, figs. 131–32 ; Groves and Bullock-Webster, *The British Charophyta,* Vol. I, p. 124, Pl. XV ; Migula, *Syn. Charac. Europ.,* p. 49, fig. 41, 1898.

Monœcious. Plants 2 cm. high, forming cushions. Stem slender, 115–150 μ thick. Internodes 2–3 times the length of the branchlets in the lower half but shorter at the top. Branchlets 6, once or twice forked ; dactyls long, 2-celled, lower cell long, end-cell short, tapering, fragile. Rarely a branchlet remains simple. Primary rays
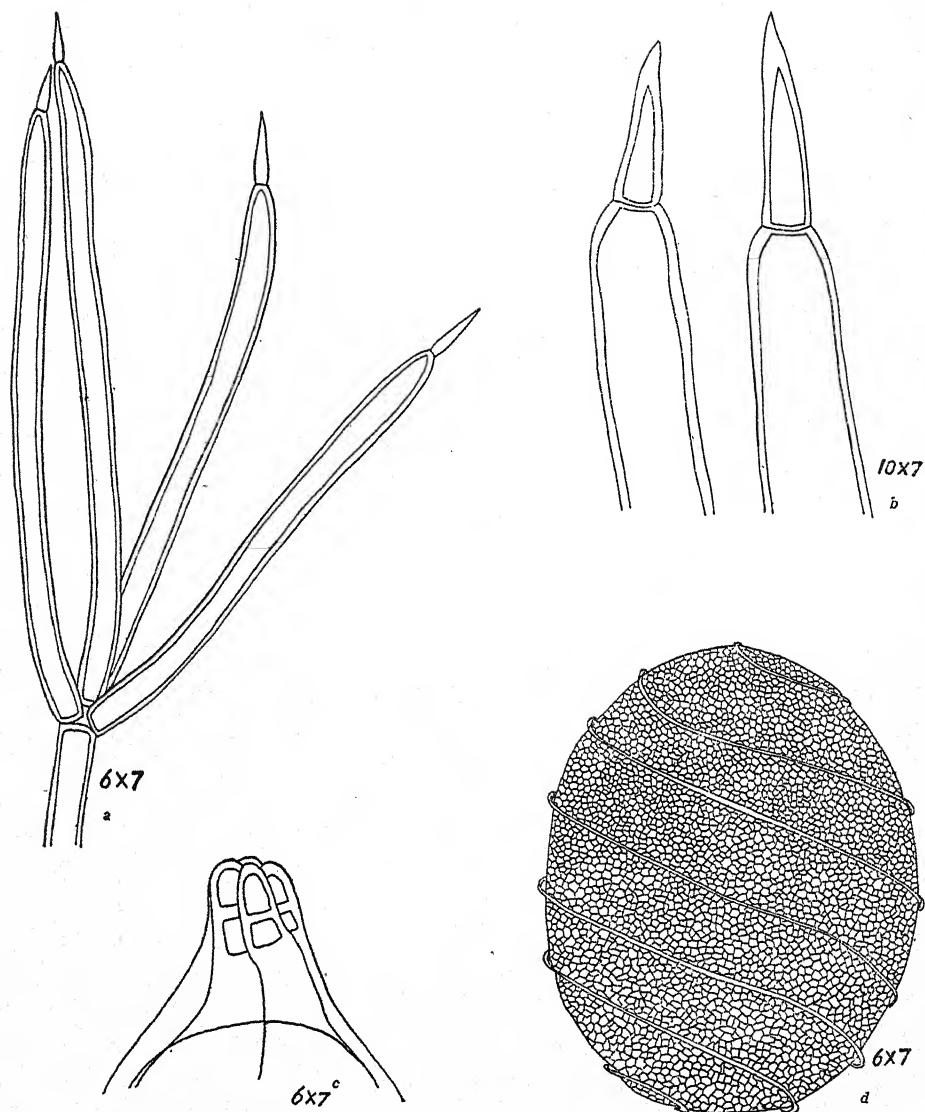
Fig. 2. *Nitella batrachosperma* Br.—(a) Dactyls (×c.225); (b) Tips of dactyls (×c.550); (c) Coronula of the oogonium (×c.225); (d) Oospore (×c.312).

short. Rays 3 at first forking; dactyls 2-3-4. Gametangia at the first furcation only. Oogonium 230–400 μ long, 150–300 μ broad, sometimes stalk-cell distinctly elongated, upper portion elongated below the coronula. Coronula 20–30 μ high. Oospore 215–225 μ long, 160–170 μ broad, ridges 6. Membrane yellow-brown, granulately reticulate. Antheridium 130–150 μ in diameter.

Locality.—Shallow drain in a railway cutting, Dudh Sagar Falls ; Sept. 1940.

The plants grew on fine mud associated with *Utricularia* sp. and *Scytonema* sp. There was slight mucus present at the growing apices of the plants.

Our plant approximates *Var. minor* but differs from the typical specimen of the species by lesser number of branchlets and rays. It has resemblances with a synonymous species *Nitella confervacea* Br. [Migula, Characeen in *Rabenhorst Kryptfl.* (1897), p. 182.] in some branchlets remaining simple, in the number of the branchlets and in its oospore. The lesser number of branchlets appears to be a constant character in some specimens so far reported from this country. Groves (1924) remarks, "A doubtful plant collected by Prof. Agharkar in 1912, in Kathiawar, agrees with *N. batrachosperma* in having the branchlets usually only twice forked, with gametangia produced at the first forking, but has *six* branchlets in a whorl, and lacks the characteristic *broad flanges* of the oospore ridges." These remarks hold good for the specimen described above. The plants described by Pal (1932) have also commonly six branchlets. On the other hand, the specimens collected by Allen (1925, 1928) from Gonda and Saharanpur had typical eight branchlets and the oospore ridges had conspicuously broad flanges. Migula (1898) mentions this species seldom having 6–7 branchlets and oospore ridges without characteristic flanges. The presence of slight mucus on our plants is also noteworthy as its presence is doubted. Our plant further differs by fewer rays and dactyls and the oospore membrane tending towards reticulated type as Groves and Bullock-Webster have noted in their *British Charophyta* (p. 125).

Nitella batrachosperma is one of the smallest species of the genus. It is related to *N. tenuissima* from which it differs by its size, by its fruiting at the first forking only, by shorter internodes, lesser furcations, in its design of the oospore membrane and having a little *neck-like elongation of its oogonium* below the coronula.

4. *Nitella oligospira* Br. Braun and Nordstedt, *op. cit.*, 1882, p. 67, Taf. V., Figs. 135–36. *Montasber der Berl. Akad.*, 1858, p. 357, Taf. II, Figs. 50–52.

Monœcious. Stem 250–620 μ thick. Branchlets 6 in a whorl, 2–3 times furcate. Primary rays $\frac{2}{3}$ the entire length of the branchlet ; secondary rays 4 ; tertiary 2–3, of which some simple ; dactyls 2–3, unequal, divergent, 2-celled, some much shortened, end-cell acuminate. Gametangia borne at first and second furcations. Oogonia solitary, 650–670 μ long (incl. coronula), 480–500 μ broad. Convolutions 8. Coronula 50 μ high. Oospore spherical, 365 μ in diameter, ridges 6, membrane reticulated, yellow-brown in colour. Antheridium 215 μ in diameter.

Localtity.—Pond, Borivali—Bombay ; March 1931.

The plant is variable in the branchlet rays. In some specimens secondary rays were 5–7 at the first furcation and the tertiary 4. The dactyls were quite short in some instances but in other few cases they were remarkably long for the species. Rarely a proliferous shoot was seen growing out from the first furcation of the branchlet.

The author agrees with Groves and Allen (1927) that the species is rather ill-defined. It comes near *N. flagellifera* Groves and Allen but it differs in the final node of the branchlet not being fertile and having no regular proliferous shoots at the first and the second branchlet nodes. The species is also allied to *N. patula* Groves and Allen but differs in having abbreviated dactyls and the marking of its oospore membrane. According to Groves and Allen *N. flagellifera* and *N. patula* may be found reducible to varietal rank on revision. *Nitella dictyosperma* H. and J. Groves is another allied species to *N. oligospira* but differs from it in never having the ultimate rays regularly shortened. *N. abyssinica* Br. and *N. microglochin* Br. show very minor differences from *N. oligospira* and are undoubtedly quite related species.

It may be noted that all these species mostly differ from one another in the length of the cells of the dactyls and their specific characters are none too convincingly distinct. In fact Braun's species require re-grouping and reductions.

The following species are noted for their new and wide localities :—
1. *N. furcata* Ag., Igatpuri, October 1940. 2. *N. dualis* Nordst, Londa; Dijapur (Parandekar), December 1940 ; Castle Rock, Dixit, September 1939 ; Shende, December 1940 ; found along with *Batrachospermum*. 3. *N. hyalina* Ag., Watrak River, Mahemdabad, May 1940 ; Small form with mucus.

CHARA

1. *Chara Braunii* Gmel. (= *C. coronata* Br.). Charac. Ind. Orient. in *Hook's Journ.*, 1849, p. 295 ; Migula, *op. cit.*, 1898, p. 72, Figs. 68–69 ; Groves in *Journ. Bot.*, XXII, p. 3, t. 242 (1884).

Monœcius. Plant entirely ecorticate. Stem 600–650 μ thick, lime coated. Stipulodes in a single circle alternating with the branchlets, long, acuminate. Branchlets 8–10, incurved, of 4–5 segments each, having a cluster of 2–3 short cells at the tip. Bract cells 5, long, acuminate. Oogonia solitary or geminate, produced at the first or the second node but not at the base, 600–650 μ (incl. coronula) long, 350–380 μ broad. Coronula 115 μ high, 230 μ broad, spreading. Convolutions 8–9. Oospore 460 μ long, 225 μ broad, black ellipsoidal. Antheridium 250–300 μ in diameter.

Locality.—Quarry, Wanowri—Poona, April 1932.

This species is variable and as many as nine forms have been described by Allen (*American Naturalist*, XVI, pp. 358–369, t. 4, 1882) from America. Braun has described *Var. Coromandelina* in *Hooker's Journ. Bot.*, 1849. It differs from *C. corallina* Willd., *C. succincta* and *C. macropogon* by *not* producing the gametangia

at the base of the branchlets. It resembles *C. nuda* Pal and *C. pashanii* Dixit but it is distinguished by well-developed stipulodes and by having a cluster of 2–3 short cells at the tips of the branchlets.

2. *Chara contraria* Kütz.—Braun and Nordstedt, *op. cit.*, 1882, p. 141 ; Migula, *op. cit.*, 1898, p. 96, Fig. 84–89. Groves and Bullock-Webster, *op. cit.*, 1924, Vol. II, pp. 36–40, Pl. XXXIII ; Sluiter in *Bot. Zeit.*, LXVIII, p. 125, t. IV, Figs. 1–5, text-figs. 1–9, 1910.

Monœcius. Stem 550–600 μ thick, corticate ; cortex diplostichous. Spine-cells solitary, inconspicuous. Primary cortical cells of the stem more prominent than the secondary. Stipulodes in two circles, small, irregular, rounded. Branchlets 8, long, spreading, of seven segments, upper 2–3 segments ecorticate. Bractcells 5, long. Oogonium solitary, 650–880 μ (incl. coronula) long, 500 μ broad. Coronula 115 μ high, 225 μ broad. Convolutions 14. Oospore ellipsoidal, black, 650 μ long, 350 μ broad. Antheridium 350–400 μ diameter.

Locality.—Canal, Barda Hills—Porbundar, Kathiawar.

The species resembles *C. vulgaris* in appearance but it is distinguished by its primary cortical cells being more prominent and large. Oospore membrane of *C. vulgaris* is tuberculate while that of *C. contraria* is granulated.

The following species are noted for their new and wide localities :— 1. *C. corallina* Willd., Alnavar (Shende), December 1939. 2. *C. gymnopitys* Br., Khandala, September 1940 ; on rocky pools with *Isœtes coromandelensis* and *Bulbochetæ*; Kolhapur (Parandekar), November 1940. 3. *C. zeylanica* Willd., River Sabarmati, Ahmedabad ; River Watrak, Mahemdabad ; River Saraswati, Sidhpur ; May 1940.

SUMMARY

1. Altogether six species of Charophytes are described in this paper, of which two are additional records.

2. New and wide localities are noted for other six species.

ACKNOWLEDGMENT

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REVIEW

" **The Common Grasses of the United Provinces,**" by Dr. N. L. Bor, M.A., D.Sc. (Edin.), I.F.S., F.L.S., F.N.I., Forest Botanist, Forest Research Institute, Dehra Dun, published by the Manager of Publications, Government of India, Delhi, in the Indian Forest Records (New Series) Botany, Vol. II, No. 1, on the 25th April, 1941.

The book embodies an illustrated account of 92 species of the commonest grasses of the United Provinces with notes on their ecology and economic uses. The text of 222 pages is illustrated with numerous fine sketches contained in 64 plates. This work has been written, as noted in the *foreword*, at the request of the Forest Department of the United Provinces who wanted to have a handbook of the common grasses of the United Provinces in order to enable their forest officers to identify and have sufficient knowledge about the utility of the common grasses found in the forests, Taungyas and plantations of the province. In this book an elementary and an advanced keys as well as a section on the morphology of the grass family have been provided much to the advantage of those with some training in systematic botany and non-botanists. Non-technical as well as scientific descriptions of all the genera and the species and the two keys—one for the beginner and the other for the more advanced students of botany will be helpful not only to the forest officers but also to the non-botanists in naming U.P. grasses dealt with in the volume. In the introduction the author has discussed in brief the occurrence of the different species of grasses under different ecological conditions. The grass-lands of U.P. also received his due attention. As an experienced forest officer and field botanist Dr. Bor's comments on the problems of rotational and periodical grazing, introduction of exotic grasses and nature of different grass-lands in U.P. are valuable information to those interested in the gasses of U.P. His hints on the practical study of grasses and dissections of the complicated parts of the spikelets of different species of grasses will be very helpful to the forest officers and botanists. The artificial but clear-cut dichotomous keys show that they have been prepared with sufficient care and accuracy. Such keys have rendered the work of determination of the grasses of U.P. an easy task. The scientific keys for the advanced systematists indicate his capacity for masterly treatment of the difficult family of Gramineæ. In drawing up the keys and notes on the tribes and the genera the author has adhered closely to his previous work on the grasses of Assam published in Vol. 5 of the Flora of Assam, 1940. In the appendix 40 species of grasses recorded in Hooker's Flora of British India have been mentioned indicating the recent changes in the names. This list will be of some help to the taxonomists in the

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study of grasses of the different parts of India. Dr. Bor has delineated, as illustrated in the case of the common Sabai grass of U.P., the method of finding out the modern specific names corrected in the light of the latest International Rules of Botanical Nomenclature. The importance of adhering to the International Rules of Botanical Nomenclature has thus rightly been emphasized. It is expected that all systematists and taxonomists in India should follow the example set by Dr. Bor in his recent works on the systematic botany of India. Index to the local names and the list of the improtant literature on agrostology of India are useful additions.

It may, however, be pointed out that the generic descriptions detached from the specific descriptions necessitated the author to repeat shorter generic descriptions for the genera having more than one species under them. This duplication of generic descriptions might have easily been avoided by describing the genera along with the species. Such a step would not have taken away the mind of the readers from the detailed descriptions of the genera under which the species have been described. It would have been of considerable value to the Indian systematic botanists and taxonomists if such a work would have contained the majority of the grass flora of a province like the United Provinces, which extends from the plains to the higher levels in the Himalayas and includes within it areas of varying climatic conditions where quite a large number of species have been reported to grow. One feels also the absence in such a work an index to all the specific names mentioned in the book.

In spite of such negligible failings in the work " The Common grasses of the United Provinces " has advanced our knowledge of the grasses of this part of India to a great extent. Such a work is a valuable supplement to the grass volume of the Flora of British India where both the genera as well as species of grasses require thorough overhauling with reference to our recent knowledge regarding the habitats and the systematic position of the Indian species of grasses and in the light of the important changes made in the nomenclature of the grass flora of India. The excellent illustrations accompanying the book have been executed with great care and reproduced faithfully. There are very few typographical mistakes and other errors in such an exhaustive work. The printing is quite good. Such illustrated works on the common grasses of other provinces in India will be welcome additions to the *Flora Indica.* Dr. Bor's work has removed a long felt want in this direction and fulfils in all respects the purposes for which this book has so ably been written.

K. BISWAS.

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Contents of this Number

A NEW SPECIES OF THE GENUS *RAPHIDIOPSIS* FRITSCH AND RICH (*RAPHIDIOPSIS INDICA* SP. NOV.) EXHIBITING MORPHOLOGICAL VARIANTS

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(*Department of Botany, Benares Hindu University*)

(Communicated by Y. Bhâradwâja)

THE genus *Raphidiopsis* (the species being *R. curvata* F. & R.) was established by Fritsch and Rich (1929) as a result of the determination of a collection of algæ made by E. Young from two small water-tanks in Newlands, West Africa. Since then another species of the genus (*R. mediterranea* Skuja) has been described by Skuja (1937, pp. 23–24, Tafel 1, Fig. 5) from Kastoria Sea, District Macedonia, Greece. It is now for the first time that the genus is being recorded from India.

The plant was collected during the course of a study of the algal plankton of some ponds and tanks of Benares City, and considerable interest was aroused by noticing certain variations in its body. This organism was a fairly constant constituent of the plankton in the Botanical Garden pond of the University and also in Durga Kund, an artificial tank, some two miles from the University premises, during the summer and winter months. In December, 1939, and May and June, 1940, it was observed that the alga became most dominant, representing $98 \cdot 5\%$ of the total algal population present in the plankton. During the above-mentioned period its growth became so prolific that it formed a thick dark-green water-bloom, not different from one produced by the species of *Euglena*.

The plant consists of isolated trichomes, which are straight as well as curved in some cases, wound in a circular form, sometimes only into a semi-circle and occasionally into a sigmoid curve (Fig. I, 1–6). The majority of the trichomes are short and composed of about a dozen cells, but occasionally longer ones are also found. Typically both ends of the trichome terminate in a point (Fig. I, 1 and 7); but during the course of vegetative reproduction these trichomes, especially those that are very long, break in the middle to form shorter ones of which one end is rounded and the other acuminate (Fig. I, 2). Frequently these trichomes break again and this process is repeated, with the result that shorter trichomes with both ends rounded as well as one-celled trichomes with one end rounded and the other prolonged into a long bristle, (Fig. I, 3 and 8 respectively), are produced. In few cases, however,

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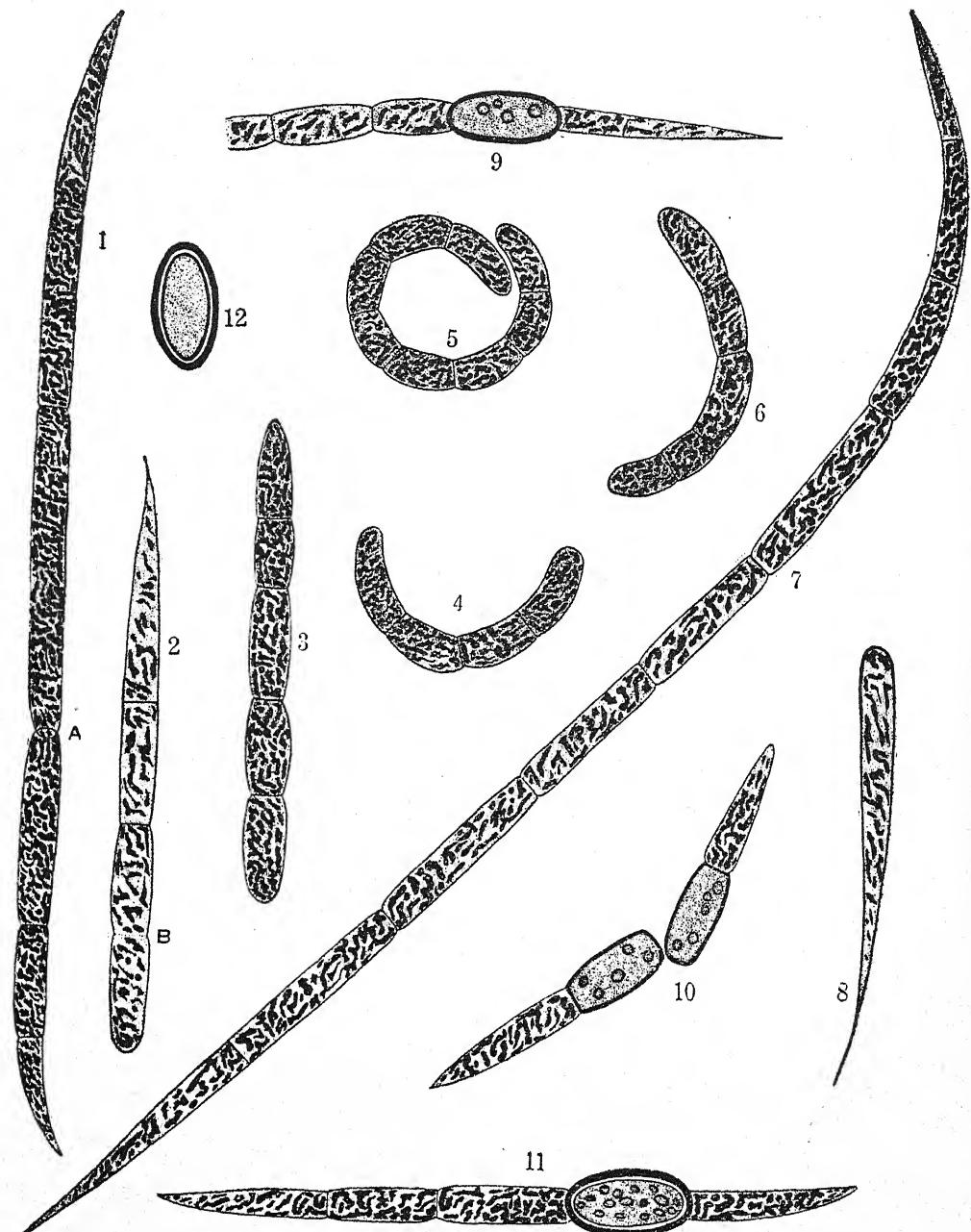
Fig. I. *Raphidiopsis indica* sp. nov. 1, a mature trichome with pseudo-
vacuoles and tapering ends, showing the place of breaking at A; 2, the
resulting trichome after breaking off (1) with one end rounded and the
other acuminate, showing division of the elongated cell at B; 3, trichome
with both ends rounded ; 4–6, semi-circular, sigmoid and circular trichomes
with both ends rounded; 7, a long trichome with both ends tapering, showing
elongated cells with pseudovacuoles ; 8, one-celled trichome with one end
rounded and the other ending in a long bristle ; 9 and 10, portions of tri-
chomes, showing subterminal immature spores ; 11, a trichome, showing
a subterminal mature spore ; 12, a parennating spore. (All × 3300).

one-celled trichomes also result by the irregular breaking of the ordinary trichomes. Trichomes with both ends rounded have been found to curve and take up gradually a sigmoid, semi-circular or circular appearance (Fig. I, 4–6), the last one reminding the habit of a typical *Anabœnopsis* but without heterocysts. In cases where the trichomes are tapering they generally commence to do so only a little distance from the extreme ends, sometimes not until the last cell is reached. The sharp end is prolonged to a variable length, and may sometimes be curved a little to one side.

The cells are constricted at the septa and usually densely filled with pseudovacuoles of irregular shape. Occasional threads without the pseudovacuoles are also met with. When the pseudovacuoles are present, the septa are generally indistinct, but they become clear after staining with iodine. They measure $1 \cdot 8$–$2 \cdot 6 \mu$ in breadth and are six to eight times as long as broad.

The spores are formed by the unequal division of the apical cell (Fig. I, 9–11), the daughter cell away from the apex being smaller than the other. The smaller cell gradually transforms itself into a spore by the usual method described by Fritsch (1904) in *Anabœna Azollœ* Strasb. and by Bhâradwâja (1933) in *Cylindrospermum muscicola* var. *kashmirensis*. The spores have never been found to be produced in the middle of a trichome as in *Raphidiopsis curvata* and *R. mediterranea*. They are formed singly (Fig. I, 9–11), in contrast to the paired ones in these two species of the genus. The mature spores are ellipsoidal with rounded ends (Fig. I, 11 and 12), being $3 \cdot 3$–4μ broad and $7 \cdot 8$–$9 \cdot 2 \mu$ long. Each spore has two walls, the outer one being very thick, smooth and brown, and the inner thin and transparent. The spores contain a few very conspicuous granules of large size (Fig. I, 9–11).

The Benares alga, therefore, differs from the African form, *Raphidiopsis curvata*, in the trichomes being narrower and usually straight with constrictions at the septa, and in the spores being smaller, subterminal, always single, and ellipsoidal. It contrasts with *R. mediterranea* in the presence of constricted septa and pseudovacuoles, and in its single spores being slightly smaller, subterminal, and ellipsoidal. The present alga may therefore be considered a new species of the genus *Raphidiopsis* Fritsch and Rich, to be named as *Raphidiopsis indica* sp. nov.

Apart from its being a new species of a little-known alga, the chief point of interest lies in the fact that during the course of its life-cycle the alga passes through four different forms :—(1) straight trichomes with both ends tapering (Fig. I, 1 and 7), (2) straight trichomes with one end tapering and the other rounded (Fig. I, 2), (3) straight trichomes with both ends rounded (Fig. I, 3), and (4) curved trichomes with rounded ends (Fig. I, 4–6). These forms that are respectively termed as variants I, II, III and IV, have been found to be seasonal in distribution. This aspect of the form-variation of the alga was studied during the year 1939–40. Plankton samples were collected from the surface water of the Botanical

Garden pond and Durga Kund at fortnightly intervals.   Each sample was examined systematically and each of the first hundred specimens of *Raphidiopsis indica* was allocated to its class, variants I, II, III and IV.   In a few cases, on account of their rarity, it was not practicable to count more than fifty individuals, and for this same reason it was not possible to obtain counts from February to April 1940.   The results as obtained are shown graphically in the following figure (Fig. II).
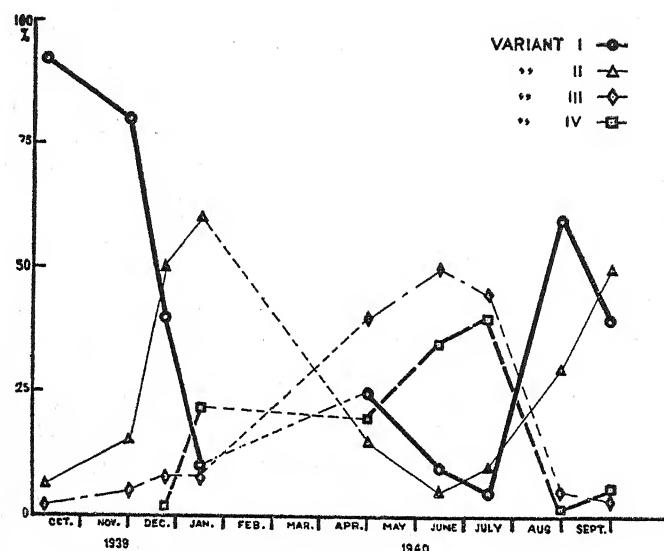

Fig. II. Graphs showing the percentage of each variant plotted against time. Each point gives the value for a particular variant as a percentage of the total number of individuals of *Raphidiopsis indica* present in the sample.

It may be seen from Fig. II that in the beginning of the winter of 1939–40, the variant I was clearly dominant; this was also the case in the middle of the rainy season.   The number of the variant I, however, fell rapidly at the end of January and again at the beginning of the rainy season of 1940.   The proportion of the variant II, however, increased at the end of January 1940.   Several cases were found where variant I was breaking up to form variant II and also giving rise to variant III, which became dominant in June 1940.   This rise in the number of the variant III was closely followed by a fall in the number of the variant II and a parallel rise in the number of the variant IV.   The latter behaviour may be explained by the fact that the variant IV, at this time of the year, was formed more by the irregular breaking of the variant I and the immediate curving of the resulting fragments than by the coiling of the variant III.

The above account clearly shows that the different variants of *Raphidiopsis indica* sp. nov. are morphologically similar and are not in any way genetically distinct, as one variant may arise from the other. That being so, one may ascribe such changes to be due to the changes in external conditions. Variant I looks like a form of *Anabœnopsis Raciborskii* Wolosz. that the writer had observed till the time it had not produced any heterocysts (author's unpublished data). It is, therefore, probable that one may mistake variant I of *Raphidiopsis indica* for *Anabœnopsis Raciborskii* till the time the latter has not developed any heterocysts especially when both the plants occur together (*cf.* Skuja, 1937). The same may be true of variants II and III that resemble very closely certain developmental stages of *Anabœnopsis Raciborskii*. The variant IV of the present alga resembles with a typical *Anabœnopsis*-like form without heterocysts. Since the distribution of these different variants is seasonal, there is every possibility of their being taken as different plants by different workers during different seasons. The present investigation, therefore, affords a caution in the matter of systematic study of these lower plants and emphasises the importance of first examining their morphological behaviour in different seasons before taking them as new forms. Seasonal variants and habitat forms are likely to mislead those who work on purely pickled materials.

SUMMARY

A new species of the genus *Raphidiopsis* (*Raphidiopsis indica* sp. nov.) has been described.

The seasonal variation in the alga has been studied and it has been observed that there are four variants of the plant, the distribution of which varies with different seasons of the year. These variants have been found to be morphologically similar since one form can give rise to another.

In conclusion, I have much pleasure in expressing my great indebtedness to Professor Y. Bhâradwâja, for his kind guidance and criticism throughout the course of this investigation.

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Attention to the following points will greatly assist the Editor and ensure early and satisfactory publication :—

Manuscripts must be typed with double spacing and on one side of the paper only. Authors are particularly requested to revise their manuscripts carefully before submitting them to the Editor. This will reduce the corrections in the proof.

In view of the high cost of publication, contributions should be as concise as possible and all unnecessary tables and illustrations should be avoided. If the contributions are very long, the authors may be required to contribute a portion of the cost of publication.

Names of genera and species should be underlined and will appear in italics. The names of the authors of genera and species should always be given

Original papers must conclude with a summary, drawing attention to the main facts and conclusions. References to literature cited should, as far as possible, be complete and must be carefully verified. A bibliography should be given at the end of the paper arranged alphabetically under authors' names.

References to literature in the text should be made by quoting the author's name and the year of publication adding the page where possible, thus (A. B. 1934, p. 25). When the author's name occurs as a part of the text, only the year and page need be given. No references should be given as footnotes.

Illustrations in line.—These should be drawn boldly in Indian ink on Bristol board or smooth white card. All necessary shading must be done in well-defined dots or lines. Colour, either in line or wash, should be avoided. It is important that such illustrations be drawn at least twice as large (in linear dimensions) as they are to appear in the reproduction. Due allowance for reduction must be made in size of lettering, thickness of line, and closeness of shading. Where possible the figures should be grouped so as either to fit the page after reduction, or to come on part of the printed page as text-figures. Authors should number the text-figures consecutively and indicate in the text places where they are to appear.

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Reviews or abstracts of books or of papers in other Journals should begin with the author's name and initials, followed by the title of the book or paper, the name, volume, number and exact pages of the serial where the paper was published, the publisher's name, the place and date of publication and preferably, in the case of books, the price.

All matter intended for publication in the Journal should be sent to the Chief Editor, P. Parija, M.A. (Cantab.), Principal, Ravenshaw College, Chauliaganj P.O., Cuttack.

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